PNEUMOCYSTIS INFECTION AND CYTOMEGALY OF THE LUNGS IN THE NEWBORN AND ADULT *

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Knowledge of pneumocystis pneumonitis has developed far more rapidly in Europe than in the United States or elsewhere in the world. It appears timely, therefore, to present the current concepts of this condition and to include a discussion of the possible relation of this infection to pulmonary cytomegaly.

Plasmacellular Interstitial Pneumonitis (Pneumocystis Pneumonitis) of the Newborn

In recent years there has been seen in central Europe a peculiar type of infantile pneumonitis which apparently does not occur in the United States. General attention was directed toward this disease when Ammich[1] in Berlin (1938) and Benecke[2] in Rostock (1939) reported a considerable number of such cases.

The disease scarcely ever occurs before the 6th week of age or after the 6th month,[3] and is evident most frequently between the 10th and 14th weeks. It appears primarily in premature or in mature dystrophic infants, in hospitals and nurseries. The incubation period is about 6 weeks.[4] Today the clinical and roentgenologic diagnosis is quite simple.[5] The mortality lies between 30 and 40 per cent. In adults a similar disease has been seen only twice: a 60-year-old female with Hodgkin's disease (Vaněk[6]), and a 48-year-old male with myeloid leukemia (Jírovec and Vaněk[7]).

At necropsy there can be seen an almost homogeneous gray-white consolidation of all lobes of the lungs. Histologically (Fig. 1), there is extensive plasmacytic infiltration of the alveolar septa (interstitial plasmacytic pneumonitis). The alveolar lumina contain either edematous fluid or a peculiar foamy material. There is almost no fibrinous exudate.

The etiology of the disease was unknown until van der Meer and

Brug,[8] Vaněk and Jírovec,[9] and Giese[10] recognized the foamy material
in the alveolar lumina as parasites. Vaněk and Jírovec, together with
most of the other authors, believed the parasites to be *Pneumocystis
carinii*, a protozoon. They spoke of pneumocystis pneumonitis. The
term *Pneumocystis carinii* had been coined by the Delanoës[11] to desig-
nate a parasite previously described by Carini.[12] Giese, on the other
hand, considered the parasites to be yeasts and the foamy structures
ascispores and ascospores. Recently it has been said[13] that it is possi-
ble to culture the parasite and to identify it as belonging to the Endo-
mycetes of the genus Saccharomyces.[14-16] The fact that therapy has
not been successful with the drugs employed against protozoa but with
those against fungi could be interpreted in favor of Giese's opinion.
The question of the nature of the parasites, therefore, cannot be con-
sidered as completely settled, although pneumocystis is most likely to
be the agent.[17] Some authors,[13-16] by inoculating the parasites into
newborn mice and cats, succeeded in producing a disease similar to
that seen in humans. As the parasite can be found also in every case
of this disease in humans, Koch's postulates would be fulfilled. One
can thus regard the parasite as the causative organism of interstitial
plasmacytic pneumonitis.

The parasites can be found in smears from the lung, in bronchial
secretions, and in histologic slides. They most often form large con-
glomerates, but occasionally lie singly in edematous alveoli[18] bordering
an area of pneumonic infiltration. One can assume, therefore, that
they occupy an alveolar lumen first as single individuals and then
multiply, using up the edematous fluid, until they completely fill the
alveolus[18] (Fig. 2). The plasmacytic infiltration of the alveolar septa
seems to appear at the same time. Using special stains, one can even
see single phagocytized parasites in alveolar septa[18,19] (Fig. 3). They
have been reported in one case in organs other than the lung.[20] Pneu-
mocystis has been found in rodents, rabbits, dogs, and sheep.[7,21,22]

In the honeycombed conglomerates within the alveolar lumina some
isolated, light-refracting "cysts" may be seen.[23] Both conglomerates
and cysts contain large amounts of acid mucoproteins and stain in-
tensely with the periodic acid-Schiff (PAS) stain.[18] They can be im-
pregnated with Levaditi's silver method[23] and stain metachromatically
with toluidin blue.[24] The cysts (sporogonia), furthermore, take Grid-
ley's[25] and Weigert's stains. They include one, two, four, or eight
Feulgen-positive clumps, which are the spores. Occasionally the dis-
integrating parasites form a granular, intensely eosinophilic material
in the center of a conglomerate.

There is a remarkable frequency of combination of cytomegalic dis-

ease and interstitial plasmacytic pneumonitis.[26-28] In a non-selected group of 200 infants, cytomegalic disease of salivary glands was diagnosed 25 times; all of these infants except 2 had an interstitial pneumonitis at the same time.[27] It seemed worth while, therefore, to consider the possibility that pneumocystis pneumonitis may occur also in adults with cytomegalic disease much more frequently than is suspected.

Cytomegaly in the Lungs of Adults

There is no doubt that cytomegaly of the lungs in adults does occur without pneumocystis pneumonitis. I myself have seen such a case in a 32-year-old male, who died of a sarcomatous type of Hodgkin's disease, mainly localized in the lungs and combined with severe cachexia. Bordering an area of pulmonary infiltration, there were numerous cytomegalic alveolar cells (Fig. 4) which were not present in other areas of the lung or in other organs. The lumina surrounded by the cytomegalic cells were empty (Fig. 5); accordingly, there was no "cytomegalic pneumonitis."

It is remarkable that there was a neoplastic disease of the hematopoietic system also in 2 other cases of cytomegalic pulmonary disease in adults (Wyatt et al.,[29] Reinhard et al.[30]). All 3 patients were severely emaciated; thus it is probable that the presence of cytomegaly in the lungs is purely accidental and a final phenomenon, as pointed out by Ackerman in Reinhard's case.

In another similar case described by Von Glahn and Pappenheimer,[31] the cytomegaly was combined with pneumonitis, but I am unable to say whether the authors considered the cytomegaly to be related to the cause of the pneumonitis.

Cytomegaly and Pneumocystis Infection in the Lungs of Adults

In 1952, Vaněk[6] reported a case of pneumocystis in a 60-year-old female with cytomegaly of the lung. In the American literature there have been reported 3 cases of cytomegalic inclusion pneumonitis in adults (McMillan,[32] Wyatt et al.[33]). As some of the photomicrographs appeared to me to be suggestive of pneumocystis infection, I asked Dr. McMillan to send me several unstained slides of his case, which he was kind enough to do. The patient was a 60-year-old Japanese female who died in Montreal in 1945. I can add nothing to Dr. McMillan's excellent case history and anatomical report and should like only to stress that, as in the infantile interstitial plasmacytic pneumonitis, all lobes of the lung were consolidated and grayish white. Microscopically, McMillan (and also Wyatt and his co-workers in their 2 cases) found, apart from the cytomegalic cells, polymorphonu-

clear cells and bacteria (*Streptococcus viridans*) as well as an eosino-
philic protein coagulum, but almost no fibrin in the alveoli.

These protein coagula most often lie in the center of the alveoli and
are surrounded by a lighter staining border consisting of numerous
round vesicles or honeycombs which can be seen under high power
(Fig. 6). Both center and border stain intensely with the PAS tech-
nique (Fig. 7). With Masson's trichrome stain the vesicles are seen to
contain very small granules. With Gridley's[25] (Fig. 8) and Weigert's
(Fig. 9) stains one can easily recognize the cysts (sporogonia) of the
parasites. I am convinced, therefore, that parasites were present in
McMillan's case and that they are identical with those found in Euro-
pean infantile interstitial plasmacytic pneumonitis (viz., *Pneumocystis
carinii*). I have not been able to recognize any regular connection
between the appearance of cytomegalic alveolar epithelial cells and the
previously described changes in the lung. The cytomegalic elements
seem to be scattered more incidentally in the lung.

As far as the histologic picture is concerned, however, there are
several significant differences between the condition in infants and
these adult cases.

1. In the adults there are often very numerous polymorphonuclear
cells in the alveoli, occasionally even occurring without parasites. In
McMillan's[32] case this seemed to be due to the simultaneous infection
with streptococci, which could be found in great numbers in the
alveoli. Sometimes one even has the impression that the clumps of
parasites have been broken up and dissolved by the polymorphonuclear
cells in the alveoli.

2. In McMillan's case[32] the alveolar septa are only very sparsely
infiltrated with polymorphonuclear cells and lymphocytes. At least
one would not make the diagnosis of interstitial pneumonitis from this
histologic picture.

3. Nevertheless, the septa in some areas are very much enlarged by
a material resembling edematous fluid under low power. Under high
power and with special stains it can be seen that this is a massive
infiltration with parasites (Fig. 10). With elastic tissue stains a
sparsity of the elastic framework of the alveolar septa is noticeable.
Therefore, I suppose that the infiltration with parasites leads to a
breaking apart of the elastic tissue and finally to its destruction.

4. In some places emphysematous alveoli can be seen, bordered
by hyaline membranes, as in the newborn (Fig. 11). Underneath the
thin membranes in the alveoli, clumps of parasites again can be found.

I do not doubt that in both cases of Wyatt and co-workers[33] the

parasites could be found in addition to the cytomegalic cells. These authors themselves identified their cases with that of McMillan[32] and I, too, find in their description the same characteristic features: protein coagula in alveoli and bronchi, sparsity of fibrin, and hyaline membranes. In addition, there can be recognized in their figures 5 and 6 the characteristic honeycomb cysts with granules. In contradistinction to the case of McMillan, there is found in both cases of Wyatt and co-workers a localized monocytic and lymphocytic, as well as plasmacytic, infiltration in the septa. The same is true also in Vaněk's[6] case.

We can thus confirm that in 2 known adult cases the cytomegaly in the lung was accompanied by a pneumocystis infection (Vaněk's[6] and McMillan's[32] cases), while it seems highly probable that in the cases of Wyatt and co-workers[33] also such a pneumocystis infection was present.

Pneumocystis Infection in Adults

There may exist in adults, however, as in infants, a pneumocystis infection of the lung without cytomegaly. In both known cases—a 48-year-old male, who died of myeloid leukemia and lobar pneumonia (Jírovec and Vaněk[7]), and a 21-year-old male, cause of death unknown (van der Meer and Brug[8])—pneumocystis infection was simply a secondary finding at necropsy.

DISCUSSION

There are in adults, clear pneumocystis infections of the lungs with pneumonitis, pneumocystis pneumonitis with cytomegaly, and cytomegaly without pneumocystis infection and without pneumonitis. With these facts in mind, we must ask ourselves whether we actually are right in speaking of cytomegalic inclusion pneumonitis and, consequently, in considering the cytomegalic virus responsible for these inflammatory changes in the lungs of adults. In McMillan's[32] case, for instance, two other organisms, pneumocystis and streptococcus, were present in addition to the cytomegalic virus, so that in this respect the case is inconclusive. In Vaněk's[6] case and most probably also in the 2 cases of Wyatt and co-workers[33] there was present, in addition to cytomegaly, a pneumocystis infection; this infection rather than the cytomegalic virus was quite probably responsible for the inflammatory changes. On the other hand, in my own case and in 2 cases from the literature, cytomegaly in the adult lung was present without pneumonitis. I find, therefore, no evidence that the cytomegalic virus alone is capable of causing pneumonitis in adults. It is

possible that the relationship is different in infants, but one must first eliminate the possibility that in cases of infantile pneumonitis with cytomegaly the inflammation may be caused by a different agent (bacteria?) than in adults.

Hitherto, I have believed that the parasites causing infantile interstitial plasmacytic pneumonitis have been confined to central Europe. Recently, however, cases have been reported in Scandinavia[34,35] and Great Britain.[36] McMillan's[32] case demonstrates that the parasite occurs also in North America, so we must consider the possibility also of an occasional fatal infection of this type in the newborn in North America.

SUMMARY

In the case of fatal inclusion-disease pneumonitis described by McMillan[32] in 1947, the same parasite (*Pneumocystis carinii*) has been found as is known to cause an interstitial plasmacytic pneumonitis in the newborn in Europe. This pneumonitis in Europe is frequently combined also with cytomegalic disease. Accordingly, as this parasite occurs in North America, one must consider the possibility of an occasional infection of this type in the newborn of that region.

It seems doubtful that there is in adults a pneumonitis due to cytomegalic infection, since a simultaneous infection with these parasites most probably was present also in the other known cases of cytomegalic pneumonitis (Wyatt *et al.*[33]).

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LEGENDS FOR FIGURES

FIG. 1. Interstitial plasmacytic pneumonitis of the newborn caused by *Pneumocystis carinii*. The alveoli are filled with apparently homogeneous material. Hematoxylin and eosin stain. × 50.

FIG. 2. Pneumocystis pneumonitis of the newborn. A bronchiole is filled with a conglomerate of parasites. Periodic acid-Schiff's (PAS) stain. × 700.

FIG. 3. Pneumocystis pneumonitis of the newborn. Parasites in the alveolar septa are indicated by arrows. PAS stain. × 900.
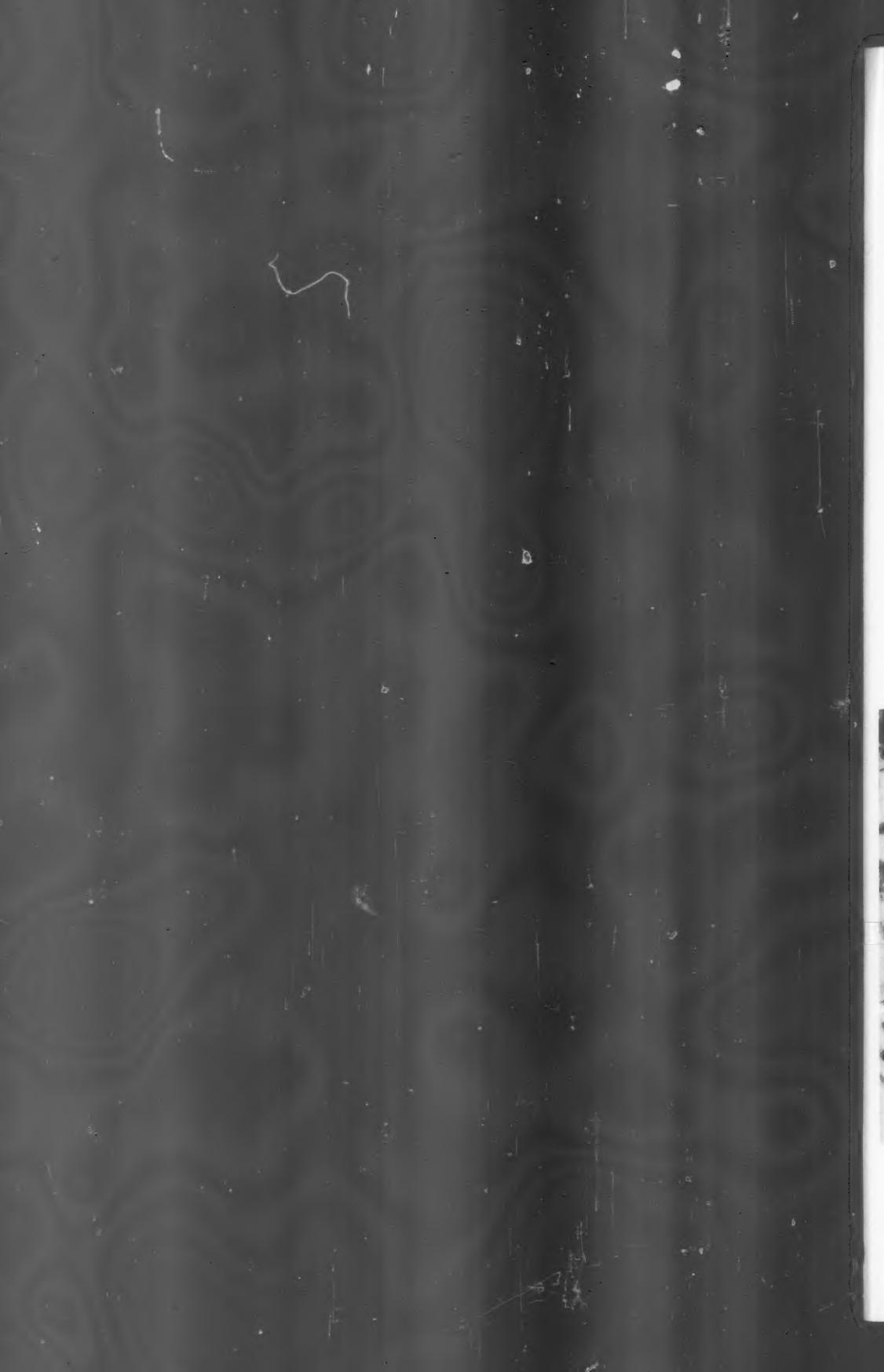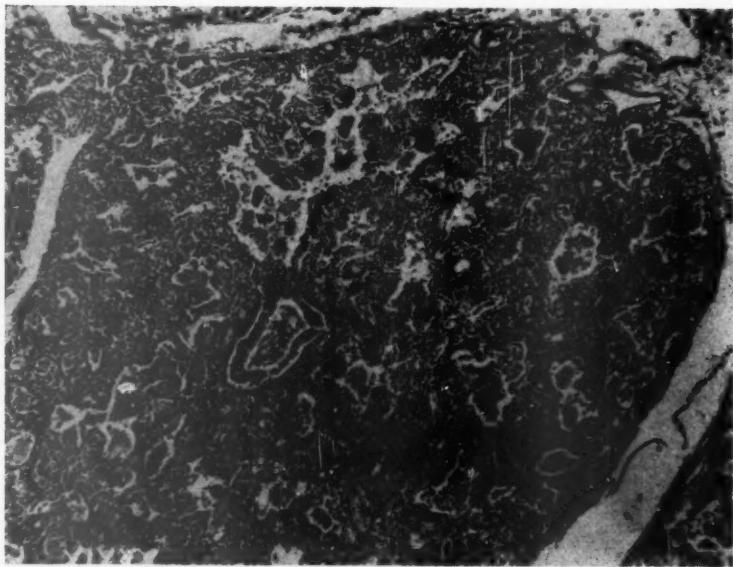
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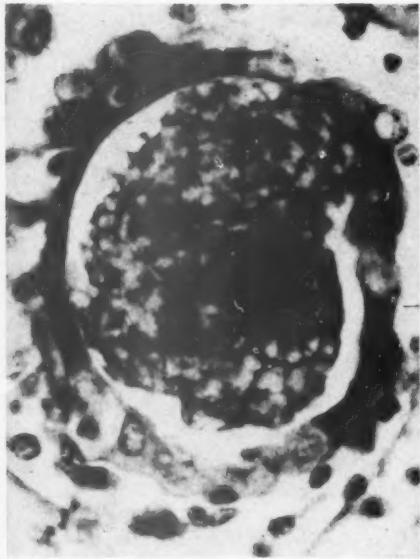
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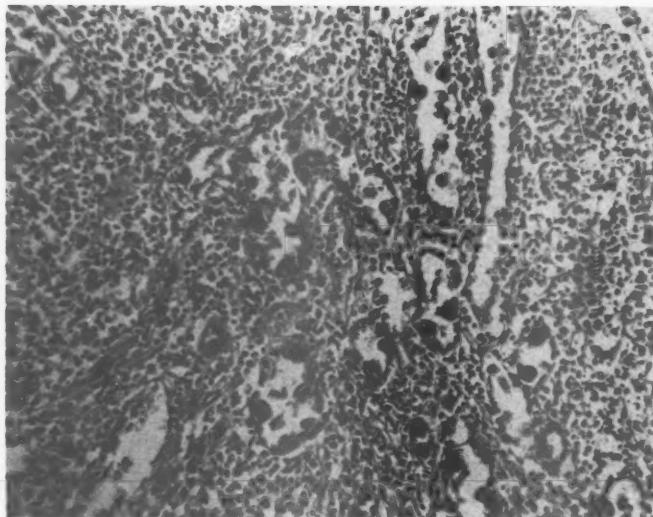
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FIG. 4. Cytomegaly of the lung in an adult with Hodgkin's disease. Hematoxylin and eosin stain. $\times$ 150.

FIG. 5. From the same case as Figure 4. $\times$ 600.

FIG. 6. McMillan's[32] case. Two cytomegalic cells at the alveolar septum. The lumen of the alveolus is filled by a conglomerate of pneumocystis. Masson's trichrome stain. $\times$ 700.

FIG. 7. McMillan's[32] case. The conglomerates of pneumocystis in the alveoli are intensely stained with the PAS method. Enlargement of the alveolar septa may be noted. (See also Fig. 9.) $\times$ 170.
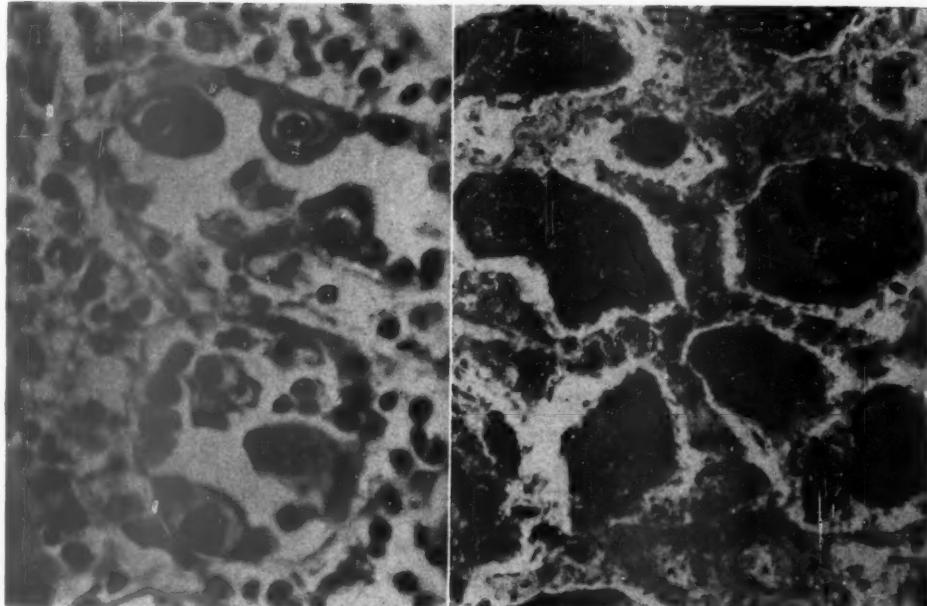
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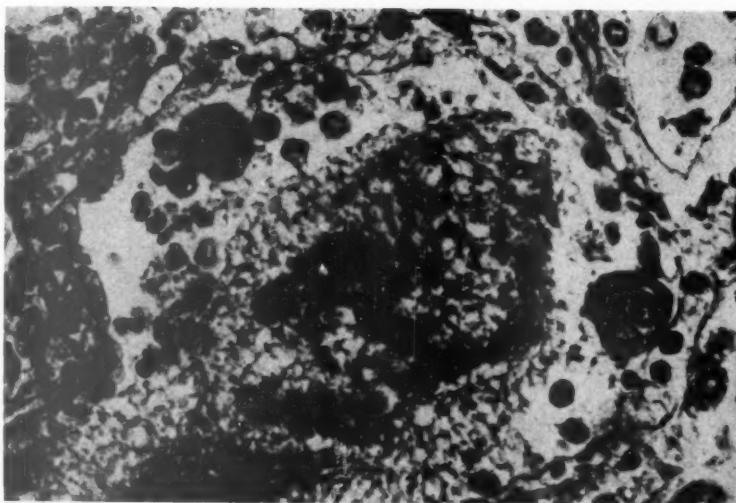
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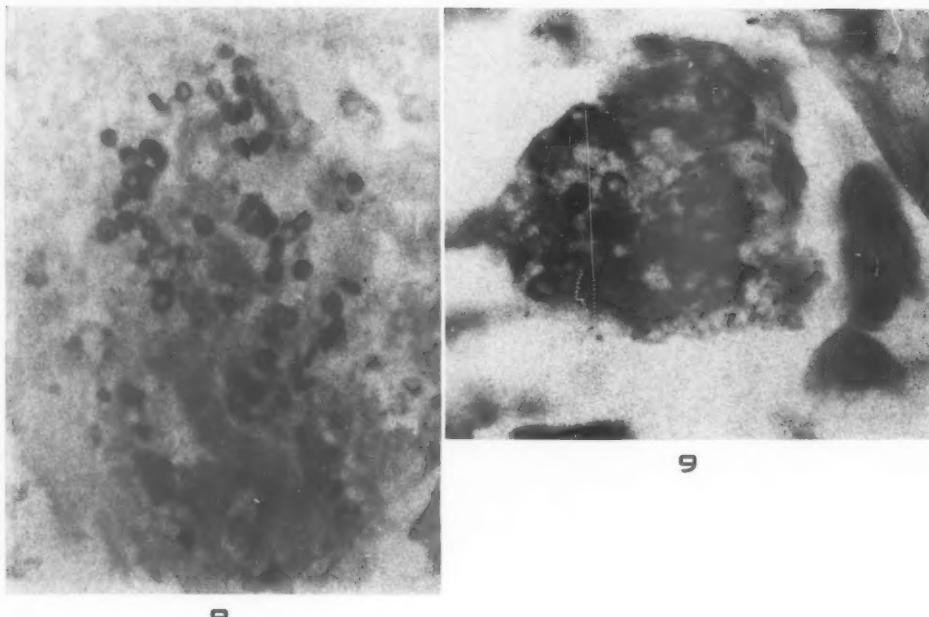
FIG. 8. McMillan's[32] case. The cysts in a mass of pneumocystis are stained with Gridley's[25] fungus stain. × 800.

FIG. 9. McMillan's[32] case. The cysts in a conglomerate of pneumocystis are stained with the Gram-Weigert stain. × 1040.

FIG. 10. McMillan's[32] case. An alveolar septum is greatly enlarged by infiltration with pneumocystis. (See also Fig. 7.) × 670.

FIG. 11. McMillan's[32] case. Hyaline membranes line the alveolar septa. Hematoxylin and eosin stain. × 50.
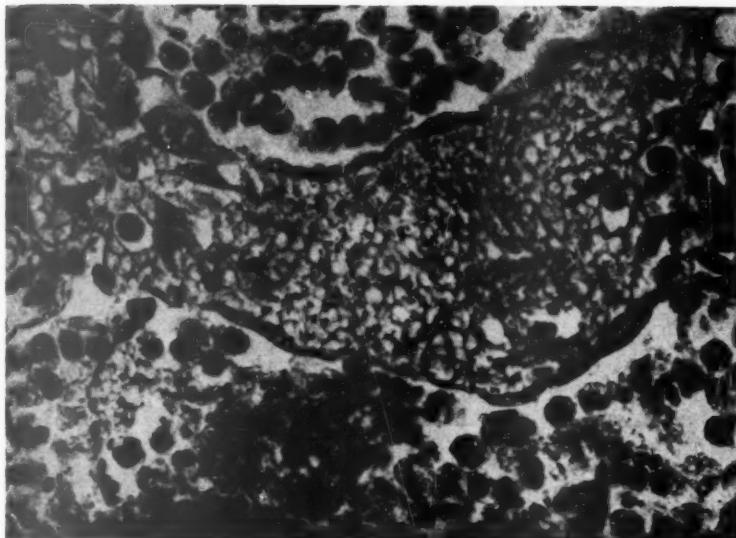
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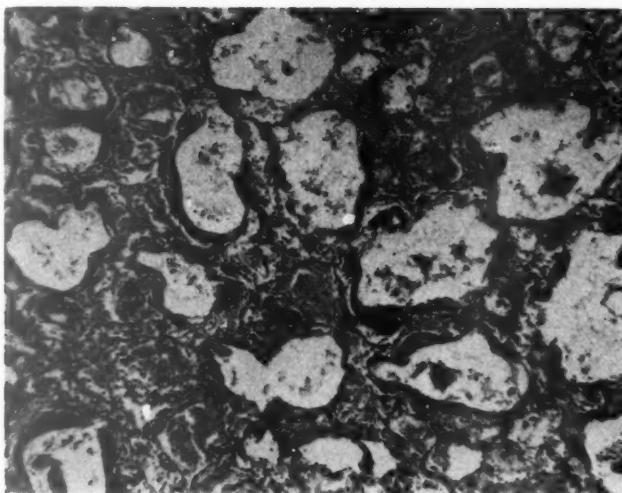
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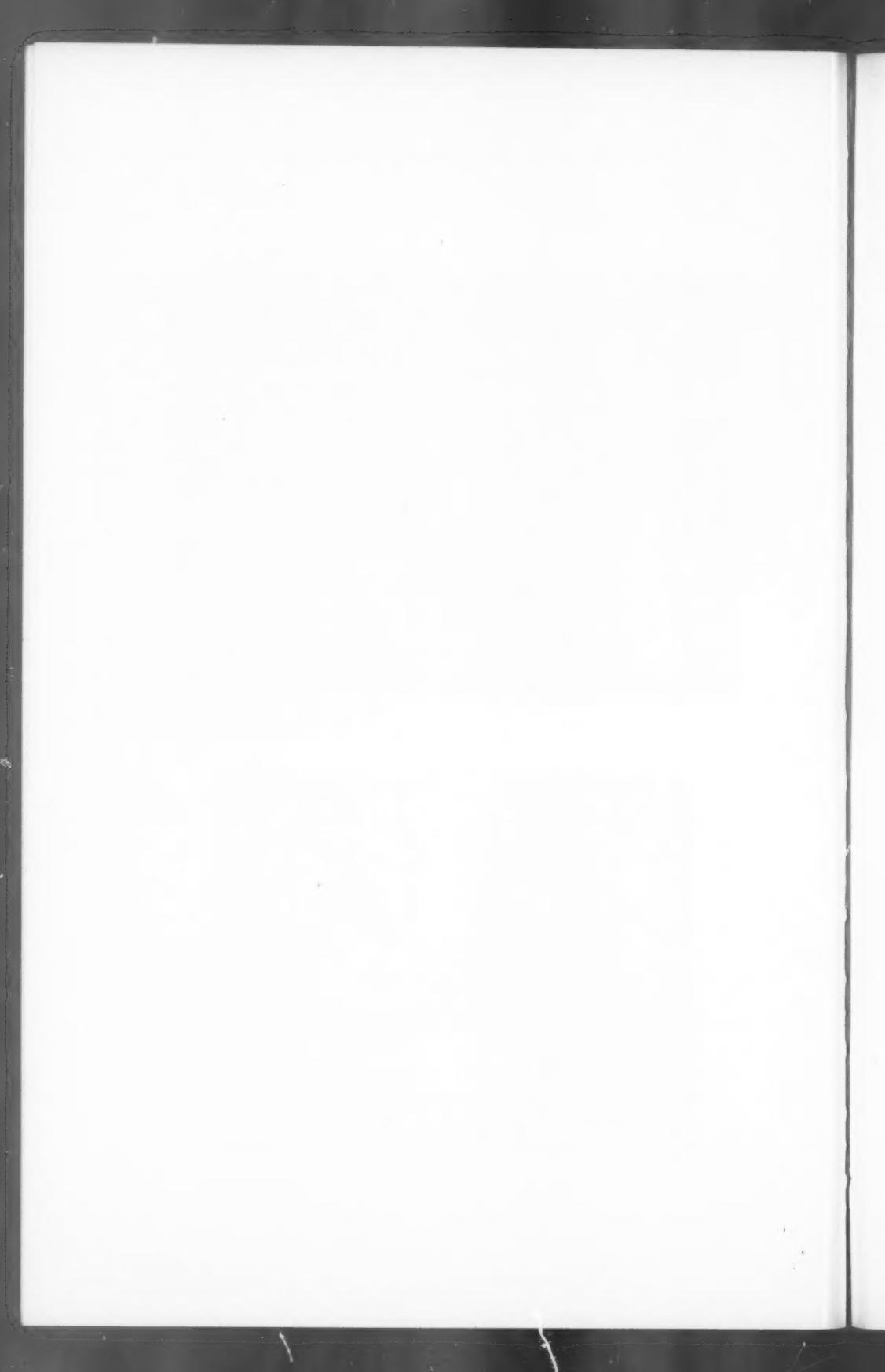
SPLENIC-GONADAL FUSION *

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Abnormal connections between the spleen and the left gonad or the derivatives of the left mesonephros have been observed occasionally. The first detailed description of such an instance was published by Pommer[1] (1889), who mentioned a similar observation demonstrated by Bostroem,[2] which apparently was published only by title. Union of the spleen with the gonadal-mesonephric structures as a teratologic syndrome was first described by one of us (W. P.[3]) in 1934, in a monograph including all cases of this entity which had appeared in the literature up to that date.

In the older reports, splenic-gonadal fusion had been undetected until recognized incidentally at necropsy. Beginning with Heitzmann[4] (1917), however, clinical recognition of the anomaly, both before and at the time of surgical intervention, has been reported with increasing frequency. The best summary of these observations in the American literature was by Emmett and Dreyfuss[5] (1943). The first successful removal of a splenic cord and attached testicle was reported by Bennett-Jones and St. Hill[6] in 1952.

The purpose of this paper is to survey all the published cases, to add 4 new cases, and to discuss the underlying embryologic and teratologic problems. The condensed observations on all cases are arranged in chronologic order. The cases in the literature with our own added total 30.

CLASSIFICATION AND SEX DISTRIBUTION

The anomaly occurs in two forms, allowing division of the cases into two major subgroups: 1. Continuous splenic-gonadal fusion in which a continuous cord-like structure connects the spleen and the gonadal-mesonephric structures; 2. Discontinuous splenic-gonadal fusion in which the fused spleno-gonadal-mesonephric structures have lost continuity with the main spleen and appear as a special variant of accessory spleen. Seventeen of the total published cases and one of our own fall into group 1 (continuous), and 9 published and 3 of our own cases in group 2 (discontinuous). The splenic-gonadal fusions, as would be expected, involved the left gonad in all cases with the possible exception of that of Gordeef and Cuenant,[26] who described a right scrotal enlargement at preoperative examination, but did not state specifically

which gonad was involved in the fusion. The sex distribution of this congenital anomaly strongly favors the male. Fifteen of the 18 cases of continuous fusion and all 12 cases of discontinuous fusion were in the male, while only 3 instances of continuous fusion were in the female. This is a male-female ratio of 9 to 1. All patients were white except the one reported by Sneath,[8] and he was a Negro.

Condensed summaries of the reported cases and of our own follow.

SPLENIC-GONADAL FUSION
A. CONTINUOUS TYPE: SUMMARY OF 18 CASES

1. Bostroem,[2] 1883. White male, age not given. Fusion of spleen and left testicle with normal location of both organs. Only title listed; no details.

2. Pommer,[1] 1889. White male, newborn. Left testicle undescended in iliac fossa, with dorsal mesorchium; right testicle in scrotal sac; cord of splenic tissue from upper pole of normally located spleen to left epididymis; splenic vessels normal; left spermatic vein received a branch from lower part of splenic cord and opened into left renal vein; a second left spermatic vein originated from testis, anastomosing with first and opening into inferior vena cava. The cord was 5.5 cm. long and 3.5 to 6 mm. wide. Other anomalies were: Perobrachius apus (partial defect of arms with rudimentary humerus only on left); complete absence of both legs and hip joints; micrognathia; atresia of anus; defect of coccyx.

3. Albutt and Rolleston,[7] 1908. Male. Splenic process bound down to posterior abdominal wall by peritoneum and extending to left scrotum.

4. Sneath,[8] 1913. Adult male, Negro. Thin splenic cord arising from upper pole, with rounded nodular portion, 2 by 1 cm., attached to left testicle and spermatic cord, almost covered by tunica vaginalis (Text-fig. 1); histologically normal spleen; the cord was 33.4 cm. long, tapering from 10 to 1 mm. in thickness. *Clinically* interpreted as a third testicle.

5. Heitzmann,[4] 1917. White male, 25 years old. Left indirect inguinal hernia, with patent canal of Nuck and fibrosed splenic cord attached to upper pole of testicle; the cord was 43 cm. long, had a nodular splenic thickening of 2.5 cm. at its lower end, contained histologically normal splenic tissue, received two arteries and one vein from abdominal wall through adhesions, traversed abdominal cavity anterior to small intestines, and disappeared below splenic flexure of colon. Found during repair of left inguinal hernia.

6. Skworzoff,[9] 1924. White male, 10 years old. Cord from lower pole of spleen, passing anteriorly to small intestine through abdominal cavity to left testicle and epididymis, enveloping the hypoplastic testicle; upper two thirds was splenic tissue, lower one third, fibrous tissue; both testicles at external inguinal ring. Other anomalies were absence of right leg except rudimentary head of femur and finger-like skin appendage; absence of left lower leg and foot, including patella; normal left femur with finger-like skin appendage in left knee area. Cause of death: typhoid fever.

7. Wiltschke,[10] 1929. White female, newborn. Splenic cord from upper pole to left mesosalpinx and left mesovarium passing ventral to the intestines. Cord, 77 mm. long, 0.5 to 2.5 mm. wide, with thickened splenic tissue in lower pole.

8. Fischer and Gissel,[11] 1935. White male, 11 years of age. Round splenic cord, about 2 mm. wide, to epididymis of left inguinal testis with hernial sac; free cord traversing abdomen anteriorly without adhesions; upper end of cord and spleen not investigated; histologically normal splenic tissue rich in lymph follicles, slight perifollicular hemorrhages, and increased eosinophils; no blood pigment or fibrosis;

slight hyaline thickening of arteries. No associated anomalies observed at subsequent operation for right inguinal testis. Found during surgical intervention for undescended testicle.

9. Kadlic,[12] 1943. White male, 19 years old. Pear-shaped mass, 4.6 by 2.2 by 1.5 cm., in left scrotum, attached by thin, fibrous cord from its lower surface to upper pole of left testicle, with a 9 cm. cord arising from its upper surface and passing through left inguinal canal; resembled spleen grossly; substantiated microscopically by presence of splenic pulp, lymph follicles, cords, and sinusoids containing

Text-figure 1. Drawing of the splenic-gonadal fusion in the case reported by Sneath.[8] (Redrawn from Sneath, W. A. An apparent third testicle consisting of a scrotal spleen. *J. Anat. & Physiol.*, 1912–13, **47**, 340–342. By permission of the Cambridge University Press.)

few erythrocytes. Upper portion of cord consisted of splenic tissue with one large artery and vein attached. Cord between spleen and testicle was fibrous, vascular, with some fatty tissue and one larger central artery and vein. Surgical specimen. Clinical diagnosis: congenital left inguinal hernia with aberrant spleen.

10. Olken,[13] 1945. White male, 56 years of age. Spleen, 200 gm., normal position; from upper pole, a splenic cord, 0.3 cm. to 0.5 cm. in diameter, passing through abdominal cavity laterally, joining left spermatic cord at inguinal ring, terminating as a bulbous mass (1.8 by 1 by 1 cm.), attached to tunica albuginea at head of left epididymis and completely covered by tunica vaginalis; accessory spleen 0.8 cm. in diameter near splenic hilus; histologically, typical splenic tissue with inflammatory hyperplasia of pulp as in spleen. Cause of death: peritonitis from perforated, bleeding, duodenal ulcer.

11. Andrews and Etter,[14] 1946. White male, 32 years old. Splenic cord with "marble-sized" enlargement at lower end of left spermatic cord; surgical exposure of testicle and cord only; 12 cm. of splenic cord which entered the inguinal canal removed; abdomen not opened; spleen not examined; histologically, normal splenic tissue in cord with slight hyperplasia of germinal centers and superficial organized thrombosed veins. Admitted with pain in left testicle and scrotum; left enlargement had been noticed since birth; particular discomfort during heavy exercise.

12. Tate and Goforth,[15] 1949. White male, 21 years of age. Splenic mass, 7 by 4 by 4 cm., attached to atrophic left testicle with tail-like process along the spermatic cord, 10 cm. removed; upper portion and spleen not investigated; abdomen not explored; histologically, normal splenic tissue attached to atrophic testis without spermatogenesis and with increased fibrous tissue and Leydig cells. Swelling first noticed at age of 3 years, after operation for left undescended testicle; mass occasionally tender since; patient well 13 years after second operation.

13. Keizur,[16] 1952 (case 2). White male, 69 years old. Bilobed spleen with beaded (alternating splenic and fibrous tissue) cord from lower pole through abdomen, joining left spermatic cord at internal inguinal ring and ending with splenic mass (2 cm. in diameter) embedded beneath albuginea in upper pole of left testicle; histologically, normal spleen with considerable free pigment, separated by thin, fibrous layer from testis with slight spermatogenesis. Cause of death: fall from tree; dead on arrival with rupture of aorta and mediastinal hemorrhage.

14. Arnett,[17] 1951 and 1953. White male, 13 months old. Tapering process from anterior margin of spleen, traversing abdominal cavity to otherwise normal left testicle, containing artery and vein and accessory spleen in midportion of the cord-like process. Sudden death with convulsion in cyanotic attack. Ectromelia with absence of right lower extremity; two upper extremity stumps, each containing only 2 cm. of humerus. Absence of left femur and fibula. Tibia attached in a ball of muscle at acetabulum. Foot contained three normal and one subnormal metatarsal bones and had four toes. Bones of pelvis normal. (Details to be published by S. D. Wu.)

15. Bennett-Jones and St. Hill,[6] 1952. White male, 10 years of age. Splenic cord (8 inches in length) from hilum of normal spleen, passing through peritoneal cavity, without mesentery. Width, 0.5 inch at upper end, narrowing in middle third to 0.25 inch, expanding at testis to 0.5 inch with little macroscopic demarcation between it and testis. Histologically, normal spleen in proximal and distal thirds of cord, increased fibrous tissue in middle third. Well defined fibrous band separated splenic tissue from main mass of testicular tissue. In one area, a group of seminiferous tubules lay on splenic side immediately surrounded by splenic tissue. Seminiferous tubules normal for patient's age; rete testis and efferent ducts distorted and compressed. Patient first seen at 7 years because of tender, enlarged left testicle; right testicle normal but retractile. Left testicle enlarged, left spermatic cord thickened. Clinical diagnosis: partial torsion or thrombosis of the spermatic cord. Returned 3 years later because of recurrence of left inguinal pain aggravated by exercise. A diagnosis of diffuse lymphangioma of the cord was suggested. Congenital hernial sac present.

16. Werthemann* and Roulet,[18] 1954. White male, 6 months old (Figs. 1 and 2). Spleen of medium size with deep incision of lower pole; 5 cm. splenic process from upper pole continuing as fibrous cord 7 cm. long, with small nodule of splenic tissue in upper portion. Cord inserted at left innominate line anteriorly. Splenic vessels normal. Normal testicles in scrotum. Other anomalies were: peromelus; slight micrognathia; absence of both forearms, hands, both lower legs, and feet; slight hypoplasia of right humerus and left femur; diagonal asymmetry of skull with prominence of right frontal and left occipital areas; congenital hydromelia; slight abnormal fissures of lungs and liver; urinary and fecal incontinence. Cause of death: bronchopneumonia. Was the second child; no family history of malformation.

17. von Hochstetter,[19] 1953. White female thoracopagus, probably newborn. Splenic cord from upper pole of spleen of left partner to slightly undescended left ovary, with two intra-ovarian splenic nodules. Blood and nerve supply to splenic nodules and cord from left ovarian artery and nerve. Abnormal fissures of main spleen. Three accessory spleens at hilus and one in gastrosplenic ligament. Other anomalies were: thoracopagus with single jejunum and upper ileum; Meckel's diverticulum with pancreas of left lower ileum; common truncus for celiac, superior mesenteric, and abdominal phrenic arteries; right umbilical artery of left partner missing.

18. Armed Forces Institute of Pathology Accession 547162. White female, stillborn (Figs. 3 to 7). Spleen 6 by 2.3 cm., in normal position with elongated process from upper pole attached to partly undescended left adnexa between tube and ovary. Process tapered to 0.5 cm. at lower end; cyst 0.5 cm. in diameter, on upper anterior portion. Tubes, ovaries, and uterus normal; no other internal congenital anomalies. Histologically, normal congested splenic tissue throughout; multiple blood supply of splenic process through hilus-like niches; fusion of lower pole of splenic process to ovary with intervening fibrous tissue; splenic cyst with capsule-like wall, mesothelial lining, and coagulated albuminous contents. Other anomalies were: hypoplasia of mandible (micrognathia); slight posterior rotation of ears; absence of both forearms and hands with nipple-like skin appendages anteriorly on each arm stump; absence of right lower leg and foot; fusion left great, second, and third toes; absence of left fourth toe. Full-term, 2,450 gm. stillborn with intracranial hemorrhage over right frontoparietal lobe; first pregnancy; no maternal diseases in first trimester except minimal vaginal bleeding.

SPLENIC-GONADAL FUSION
B. DISCONTINUOUS TYPE: SUMMARY OF 12 CASES

1. Finaly,[20] 1926. Male, newborn. Accessory spleen in left epididymis with congenital left inguinal hernia.

2. Talmann,[21] 1926. White male, 22 years old. Accessory spleen, 2.5 cm., in head of left epididymis; second fibrosed accessory spleen, 0.8 by 0.5 by 0.4 cm., on spermatic cord at external inguinal ring; histologically, splenic tissue with hyperplasia; scarring, with pigment prevailing in upper accessory spleen; old thrombosis of spermatic vein; atrophy of testicle; thickening of albuginea and tunica vaginalis; atrophy of vas. Tenderness and swelling of testicle after long marches (soldier); painful swelling of left testicle (size of goose egg) during malaria 4 months previously, receding to previous size after recovery.

3. Osselladore,[22] 1928, and Frasson,[23] 1942. White male, 19 and 30 years old at times of respective reports. Three masses of splenic tissue, largest, 4.7 by 3.9 cm., at lower pole of testis, deep red, covered by tunica albuginea with definite splenic capsule. Two smaller masses, wine red, attached to region of splenic-gonadal fusion by thin sheet of tunica vaginalis and floating free in scrotal cavity. Histologically,

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* Information contributed by Prof. A. Werthemann, Basle, Switzerland.

largest splenic mass covered by tunica albuginea and splenic capsule; some fibrosis of pulp with hemorrhage; arterial supply through capsule and its septa. Atrophic changes in testis. Left testicle about twice normal size since birth, bilobed, with two small, soft, movable nodules attached. Exploration and biopsy of the two small nodules at age 19. Left orchiectomy at age 30.

4. Settle,[24] 1940. Male, age and race unknown. Tertian malaria with painful swelling of scrotal spleen, surgically removed. Case not reported, only mentioned in discussion.

5. Emmett and Dreyfuss,[5] 1943. White male, 47 years of age. Encapsulated accessory spleen, 4 by 2 cm., attached by broad base to albuginea of upper pole of testicle and head of epididymis, surgically removed. Testicle, epididymis, and spermatic cord appeared normal; histologically, normal splenic tissue, small follicles, hyperplastic red pulp. Right inguinal hernia repaired. Preoperative diagnosis: bilateral indirect inguinal hernia with tumor of left testicle.

6. Sartor,[25] 1948. White male, 9 years old. Walnut sized accessory spleen at inferior pole of left testicle. Histologically, splenic tissue. Found during medical inspection of school children. Surgically removed.

7. Gordeef and Cuenant,[26] 1951. White male, 9 years old. Surgical specimen: upper third, semilunar in shape and resembled testicle, separated by fibrous tissue from lower two thirds which was a firm, reddish, nodular mass. Spleen was situated between testicle and corpus of Highmore and head of epididymis, pushing latter two structures laterally. Microscopically, normal spleen and immature testis. Patient first examined because of right scrotal mass. Palpation revealed a heavy testicle about five times larger than a normal testicle, with an irregular, bosselated surface; epididymis and spermatic cord normal, but vaginal process could not be felt. No lymphadenopathy. General physical condition excellent. Preoperative diagnosis: malignant tumor.

8. Keizur,[16] 1952 (case 1). White male, 4 years of age. Accessory spleen, 3 by 5 by 2 cm., in upper pole of left testicle beneath tunica albuginea; histologically normal; spleen and testicle separated by thin, fibrous layer. Left scrotal mass, 3 by 5 cm., noted at physical examination preceding operation to correct cardiac malformation. Parents stated that mass was present at birth and had not changed in size. Left orchiectomy performed 1 month after successful Blalock procedure.

9. de Cesaris,[27] 1952. White male, 13 years old. Accessory spleen, 8 by 4 by 3 cm., at upper pole of testicle; histologically, spleen with hyalinization of arterioles. Left testicle larger than right since birth, gradually increasing in size. Painful on effort. Tender to palpation. Preoperative diagnosis: tumor of the testicle.

10. A.F.I.P. Acc. 121792. White male, 24 years of age. Accessory spleen, 4.5 by 2 by 1 cm., with smooth gray capsule save for one lacerated surface, located in excised upper half of left testicle; histologically, only splenic tissue with some fibrosis of stroma and decreased pulp. Lower half of testicle appeared normal and was not removed. Recurrent attacks of epididymitis and discomfort of left spermatic cord following herniorrhaphy in civilian life several years previously.

11. A.F.I.P. Acc. 317305. White male, 23 years old. Accessory spleen, 1.7 by 2.3 cm., in upper pole of left testicle, completely encapsulated; testis, epididymis, and vas normal; dilatation of pampiniform plexus; histologically, normal splenic tissue. Mass in left scrotum since 1945, recently increasing in size without symptoms; physical examination revealed upper third of enlarged left testicle to be firmer than normal but not tender.

12. A.F.I.P. Acc. 644069. Male, 11 years old. Race unknown. Admitted for tumor of the scrotum. Surgical specimen: ovoid, deep red, encapsulated mass, 1.5 by 1.4 by 1.1 cm.; surface smooth, cut section grayish pink; removed from soft tissue between scrotal skin and spermatic cord just above and lateral to testis. Microscopically, well defined capsule with many lymphoid follicles and follicular arteries suggestive of splenic tissue; elongated sinusoidal spaces lined by endothelium and

containing red blood cells; cords separating these spaces resembled cords of Billroth. The mass was traversed by fibrous septa resembling trabeculae, which contained blood vessels similar to those of the spleen. Diagnosis: accessory spleen, scrotum.

CLINICAL OBSERVATIONS

In Heitzmann's[4] case the spleen attached to the upper pole of the testicle was an incidental finding during the repair of a left indirect congenital inguinal hernia. In other cases the aberrant splenic tissue also was found during hernial repair or operation for undescended testicle. The aberrant splenic tissue gave rise to clinical symptoms in only a few cases. Talmann's[21] patient noticed tenderness and swelling of the left testicular area after long marches. The mass also became large and painful during an attack of malaria, receding to its former size after recovery. A similar response to malaria was observed in the case mentioned by Settle.[24] Occasional tenderness of the scrotal mass, first noted at the age of 3 years, was a prominent symptom in the case reported by Tate and Goforth.[15] Scrotal tenderness was also a symptom at 7 years in the case of Bennett-Jones and St. Hill[6] and at 9 years in the case of Gordeef and Cuenant.[26] Increasing pain and discomfort, with attacks of swelling during systemic infections in the 11 years after exploration and biopsy, were the indications for orchiectomy in the case of Osselladore[22] and Frasson.[23] Recurrent attacks of "epididymitis" and discomfort in the region of the left spermatic cord were observed in one of our cases after herniorrhaphy (A.F.I.P. Acc. 121792). The presenting symptom in Andrews and Etter's[14] case was pain in the left testicle. The enlargement had been present since birth and caused particular discomfort during strenuous exercise. These observations are of interest, since pain in the left hypochondrium after running or other violent exercise often has been related to splenic engorgement with painful capsular tension.

In a number of cases the scrotal mass or masses[22,23] were observed throughout life for varying periods; sometimes they had been present since birth and were believed to represent a third testicle[8,22,23] or a testicular tumor.[5,26,27] The only preoperative diagnosis of aberrant scrotal spleen with congenital left inguinal hernia recorded in the literature was made by Kadlic[12] (1943).

Relationship to Gonadal Descent

As is to be expected, the abnormal fusion of the spleen to the mesonephric-gonadal structures frequently interferes with the normal completion of gonadal descent and orderly closure of the processus vaginalis peritonei in the male. Of the 15 males with splenic cord, only 8 showed completely descended testicles. In one case[1] a left ab-

dominal testicle was located at the iliac fossa, in one[11] bilateral inguinal testicles were combined with congenital left indirect inguinal hernia, and in another[9] both testicles were at the external inguinal ring. In 4 cases the position of the testicles was not stated. Congenital left inguinal hernias were present in 4 cases of the continuous type.[4,6,11,12] In one of our cases of the discontinuous type (A.F.I.P. Acc. 121792), a left indirect inguinal hernia was found, but it was not stated specifically to be congenital. In the case of Emmett and Dreyfuss[5] a right congenital hernia was observed.

In the instances of continuous splenic cord in the female, complete inhibition of ovarian descent was apparent in our own case (A.F.I.P. Acc. 547162) and partial inhibition in von Hochstetter's[19] case.

GROSS ANATOMICAL FINDINGS

All cases of the continuous type showed a cord of splenic tissue, appearing as if spun out of the spleen, that connected with the left testicle, epididymis, ovary, or mesovarium. The case of Werthemann and Roulet[18] was the only one in which it could be assumed that the cord terminated at the left innominate line anteriorly. In all cases in which the exact relationship of the cord to the spleen was described or illustrated, it arose from the cranial (upper) pole. Bennett-Jones and St. Hill,[6] however, described the cord in their case as arising from the region of the hilum of the spleen. In most cases the spleen was otherwise normally formed and located. Keizur[16] (case 2) described a two-lobed spleen, and von Hochstetter[19] mentioned abnormal fissuring and hilar accessory spleens. The cord usually tapered downward, often with a terminal bulbous mass of splenic tissue at the gonadal attachment. The cord was completely splenic, or partly fibrous, or beaded with multiple nodular masses of splenic tissue. Only von Hochstetter's case showed actual inclusion of two masses of splenic tissue in the ovary; Bennett-Jones and St. Hill found seminal tubules in the splenic tissue. In all other cases there was attachment only and no intermingling with the mesonephric-gonadal structures. The cord usually was seen as a free intraperitoneal structure, often traversing the abdomen anteriorly to the intestinal loops. The splenic vessels were found to be normal in every instance in which reference was made to their examination.

In cases of the discontinuous group the aberrant splenic tissue presented itself as a distinct encapsulated mass attached to the testicle or to the head of the epididymis. In Talmann's[21] case there were two and in the case of Osselladore[22] and Frasson,[23] three splenic masses in

the scrotum or on the spermatic cord. In no instance were remnants of a previously continuous cord seen on the isolated aberrant splenic tissue.

HISTOLOGIC FINDINGS

The splenic tissue of the cord as well as of the isolated aberrant masses sometimes was completely normal, although regressive changes, including fibrosis, thrombosis, calcification, thickening of trabeculae, and deposition of blood pigment, often have been noted. These changes may lead to complete fibrous replacement and formation of fat tissue in some portions of the splenic cord. In our case of continuous fusion (A.F.I.P. Acc. 547162) a peritoneal inclusion cyst was present in the upper portion of the splenic cord. Occasionally, subnormal differentiation into pulp and follicles and, sometimes, congestion of the pulp have been noted. In some cases the splenic tissue participated in the function of the main spleen, as is the rule with ordinary accessory spleens. In his case of perforated duodenal ulcer with peritonitis, Olken[13] found the scrotal spleen and also the main spleen in a state of inflammatory hyperplasia. Emmett and Dreyfuss[5] also noted hyperplasia of the red pulp.

The area of fusion is variously described as a thinned or thickened layer of fibrous tissue, occasionally enveloping the spermatic cord, the epididymis, or the testicle. The splenic tissue may or may not be completely covered by tunica vaginalis. The left testicle of Talmann's[21] patient was atrophic, fibrosed, and contained an increased number of Leydig cells. There was also old thrombosis of the left spermatic vein, atrophy of the left vas, and thickening of the tunica albuginea and the tunica vaginalis. Similar changes were noted by Tate and Goforth[15] in the atrophic left testicle of their 21-year-old patient.

The splenic cord frequently contained a sizeable artery and vein running a longitudinal course, sometimes with multiple branches entering the cord through hilus-like niches, as in our own case (A.F.I.P. Acc. 547162). The relationship of the blood vessels and nerves in the splenic cord has been described in detail by von Hochstetter,[19] who based his observations on reconstruction of serial sections. He found that branches of the left ovarian artery and nerve supplied the intraovarian splenic nodules and the lower portion of the splenic cord. In Pommer's[1] case it was noted also that the left spermatic vein received a branch from the lower portion of the splenic cord, while a second left spermatic vein arose from the left testicle. In view of these findings the origin of the vessels supplying the splenic cord deserves more careful attention.

Embryologic Findings

The splenic anlage is formed in the left dorsal mesogastrium in the fifth week, in embryos of 8 to 10 mm. length, and consists of multiple, small masses which usually fuse to form one organ.[28] It is in intimate topographic relationship to the mesonephros and the gonadal anlage until gonadal descent and mesonephric involution begin at an embryonic length of about 20 mm. in the eighth week.[29] The origin of splenic-gonadal fusions must be dated in the period of close proximity of these structures. At present, the cause remains obscure. It is debatable whether this faulty union is brought about by fusion across the separating coelomic epithelium, as most investigators assume. Von Hochstetter[19] favored the assumption that the derivatives of the urogenital fold and the splenic anlage unite by means of the caudal terminating fold, which is intimately related to the diaphragmatic pillar of Uskow. If this were the case, the coelomic space would not have to be bridged. He believed that the intimate intermingling of splenic and ovarian circulation and innervation in his case favored this explanation rather than that of mere fusion. On the other hand, the analogy with dystopic displacement of adrenal cortical nodules along the mesonephric-metanephric derivatives, including gonads, mesosalpinx and epididymis, cannot be ignored. It is open to speculation whether the discontinuous fusions are derived from an isolated hump of the splenic anlage — as some accessory spleens undoubtedly are — or whether the connection with the main spleen, originally present, has been lost, leaving no vestiges. The observations seem to favor the first assumption.

Relationship to Peromelus and Micrognathia

Special emphasis should be given a syndrome of rare malformations which appears rather frequently in combination with the continuous type of splenic-gonadal fusion. Five of the 18 cases showed severe degrees of peromelus and in 3 of these micrognathia was an additional feature. The combination of defects of the extremity (peromelus) and hypoplasia of the mandible (micrognathia) has been observed repeatedly, particularly by Gruber.[30] In this group there were 5 such cases; 4 male[1,9,17,18] and one female (A.F.I.P. Acc. 547162). In all of these, severe malformations and defects of the extremities were present. The details are given in the condensed summaries and in the schematic drawings of these 5 cases (Text-fig. 2). The common denominator for these rather heterogeneous malformations is the time of critical de-
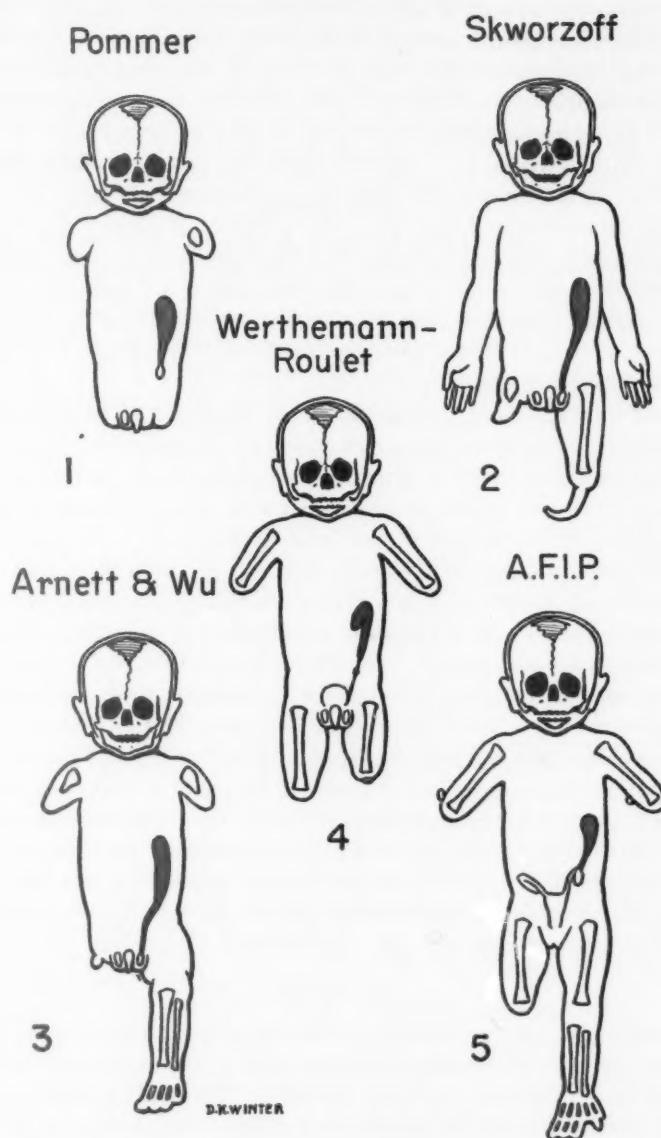
Text-figure 2. Diagrammatic sketch made from the information available on the five reported cases of peromelus associated with splenic-gonadal fusion. Micrognathia is present in the cases of Pommer,[1] Werthemann and Roulet,[18] and the A.F.I.P. Acc. 547162.

velopment. In a fetus of 17 mm. in greatest length the buds of the extremities are differentiating; Meckel's cartilage—the mold for the bony mandible—is forming, and the spleen is in intimate contact with the mesonephric-gonadal anlage. This stage of development is illustrated by serial sagittal sections of a 17 mm. embryo from our own collection (Figs. 8 to 11). A more diffuse but still not fatal injury to the fetus at this time could produce the syndrome of splenic-gonadal fusion, peromelus, and micrognathia. Recently Mushett[31] has shown experimentally on chick embryos that short periods of anoxia produce peromelus combined with malformations of eye, brain, and spinal cord. It is interesting in this connection that the patient of Werthemann and Roulet[18] showed hydromyelia of the cord in addition to splenic-gonadal fusion, peromelus, and micrognathia.

Malformations other than those included in the syndrome described above were observed in only 3 of the 18 cases of continuous fusion and in none of the 12 cases of discontinuous splenic-gonadal fusion. Peromelus was a feature in 2 of these 3 cases. In Pommer's[1] case a defect of the coccyx and atresia of the anus were present. The patient of Werthemann and Roulet[18] showed diagonal asymmetry of the skull and abnormal fissures of lung and liver. Von Hochstetter's[19] observations were made on an old museum specimen of thoracopagus without peromelus (it would appear from the picture that the extremities had been severed previously), which showed a Meckel's diverticulum, with the pancreas on the lower ileum of the left partner, a common truncus for the celiac, superior mesenteric, and abdominal mesenteric and abdominal phrenic arteries, and absence of the right umbilical artery of the left partner. This small yield of other malformations compared with the frequent combination of splenic-gonadal fusion, peromelus, and micrognathia, each rare by itself, again emphasizes that this syndrome represents an intrinsic combination reflecting the time of action of the causative factor and not a mere chance association.

SUMMARY

Twenty-six cases of gonadal-splenic fusion collected from the literature have been studied in conjunction with 4 new cases from the files of the Armed Forces Institute of Pathology. This malformation occurs in two forms: continuous, in which the main spleen is connected by a cord of splenic and fibrous tissue to the gonadal-mesonephric structures, and discontinuous, in which discrete masses of splenic tissue are found fused to these same structures.

Study of this malformation in its embryologic aspects indicates that

it has its origin between the fifth and eighth weeks of embryonic life.

Peromelus was observed in combination with splenic-gonadal fusion in 5 of the 30 cases, and in 3 of the 5 micrognathia also was present. This association of rare malformations in almost one fifth of the series constitutes evidence of a syndrome, since it occurs in too high a percentage of cases to be an effect of chance.

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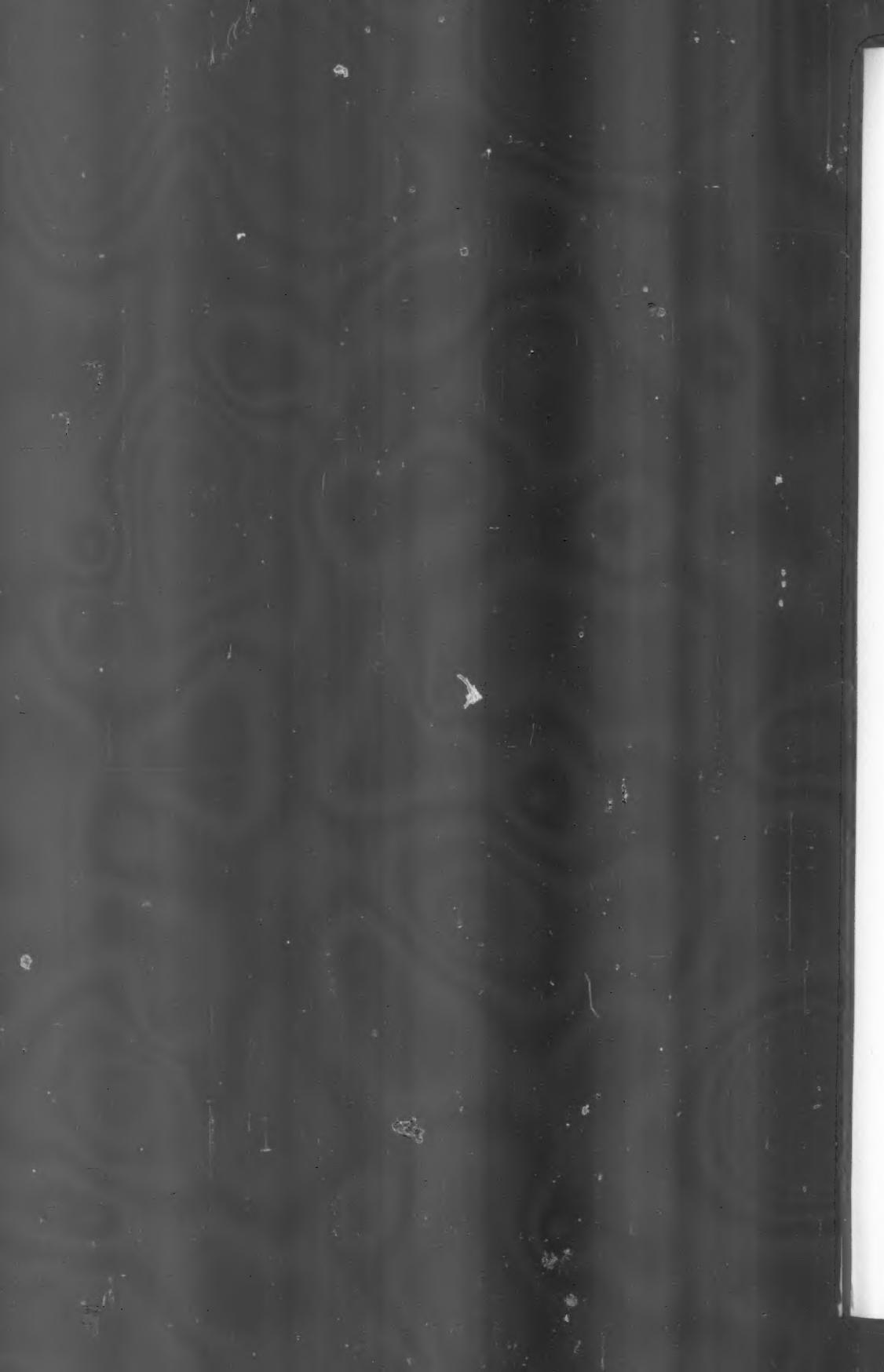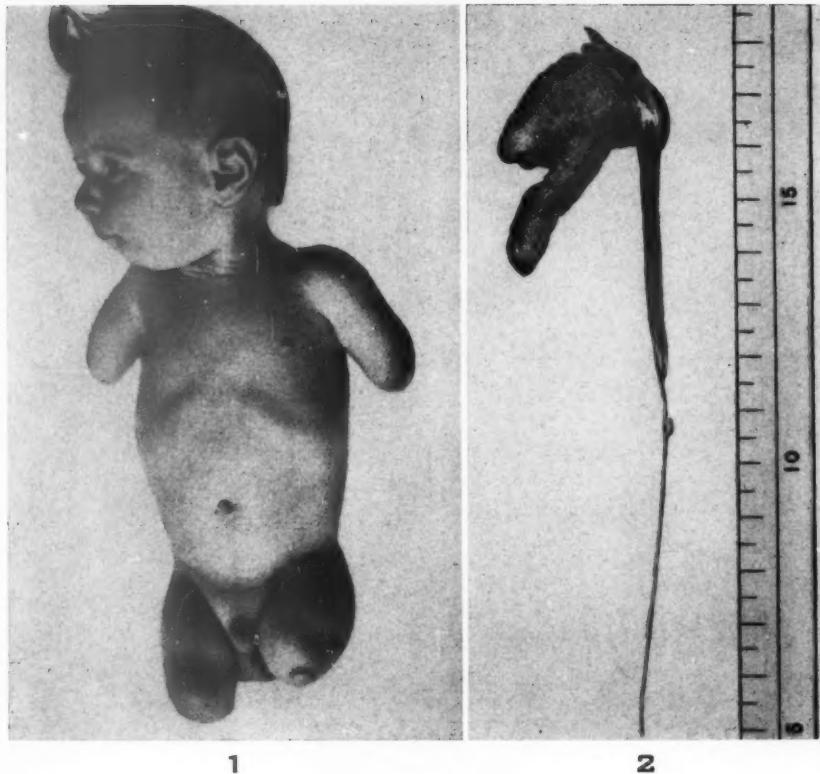
LEGENDS FOR FIGURES

FIG. 1. Clinical photograph of the case of Werthemann and Roulet.[18] (Supplied by Professor E. Freudenberg, Children's Hospital, University of Basle, Switzerland.)

FIG. 2. Splenic-gonadal fusion in the case of Werthemann and Roulet.[18] (Supplied by Professor A. Werthemann, Department of Pathologic Anatomy, University of Basle, Switzerland.)
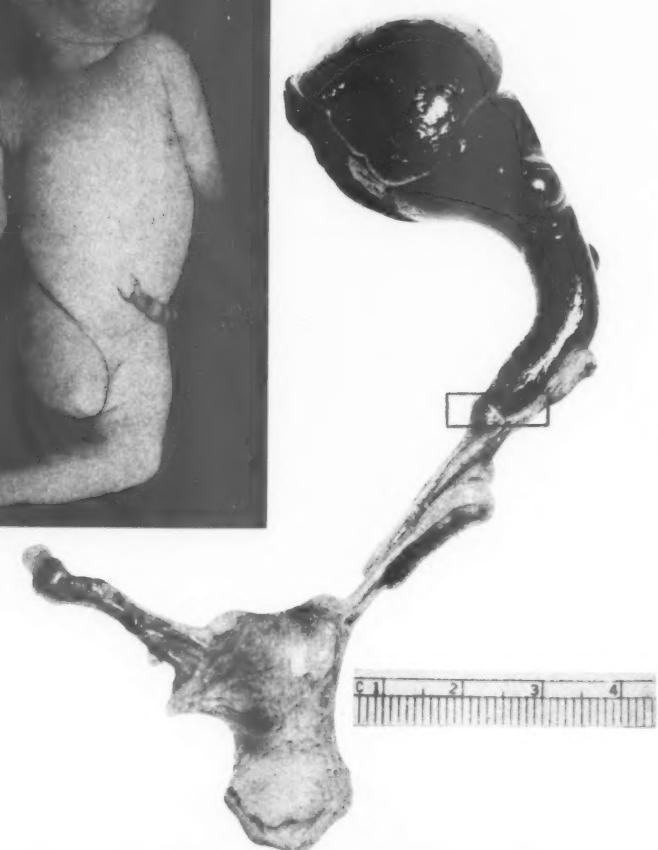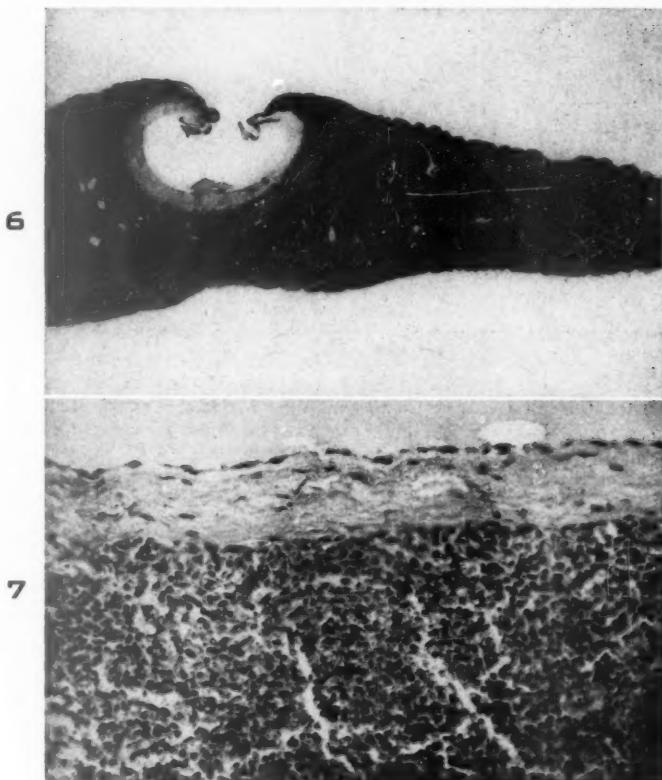
FIG. 3. Armed Forces Institute of Pathology Accession 547162, showing absence of both forearms and hands and of the right lower leg and foot (peromelus) with micrognathia.

FIG. 4. Photograph of splenic-gonadal fusion (same case as that from which Fig. 3 was taken).

FIG. 5. Section through the side of the splenic-gonadal fusion as indicated by the rectangle in Figure 4, showing the ovary on the left and the spleen on the right. × 75.

FIGS. 6 and 7. Same case as that from which Figures 3, 4, and 5 were taken. *Fig. 6.* Section through splenic cyst. × 10. *Fig. 7.* Section through wall of cyst showing cell-lining in a single layer with outer fibrous connective tissue wall. × 48.

FIGS. 8 to 11. Sagittal sections from a serially cut 17 mm. fetus from the files of the Charleston General Hospital, Charleston, West Virginia. *Fig. 8.* Developing upper extremity bud. *Fig. 9.* Developing lower extremity bud. *Fig. 10.* Developing gonad and spleen in close proximity to each other. *Fig. 11.* Developing Meckel's cartilage.
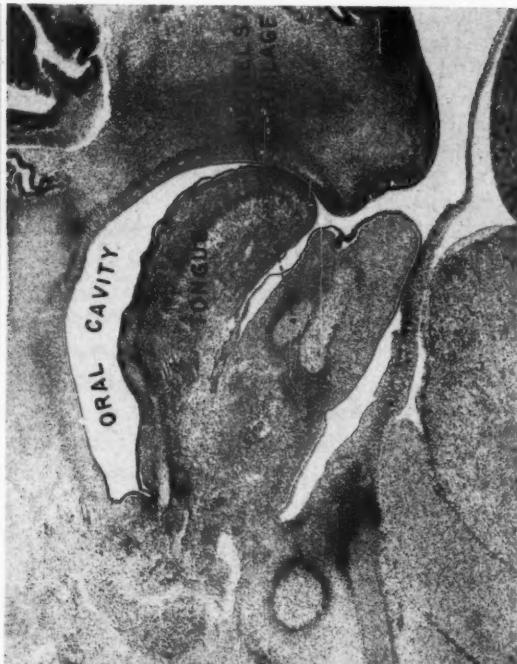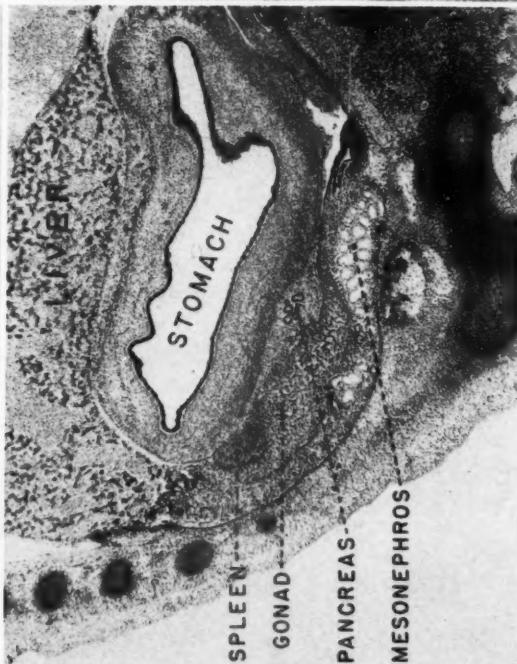
FIBRO-ELASTIC HAMARTOMAS OF HEART VALVES *

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In 1951, Prichard[1] was able to collect 28 cases of fibro-elastic hamartomas of the heart valves: 9 of the aortic, 9 of the tricuspid, 5 of the mitral, and 5 of the pulmonary valves. Raeburn,[2] in 1953, added 2 more cases: one on the mitral and one on the pulmonary valve. These congenital malformations, sometimes called papillary fibromas, are formed from tissues normally found in the heart valve but are in abnormal arrangement and are therefore properly called hamartomas. The paucity of reported cases probably results from their apparent lack of clinical significance rather than from their actual frequency.

The hearts from 50 consecutive necropsies were examined carefully to determine the incidence of both large and small fibro-elastic hamartomas. Examination of the hearts under water was found to be necessary since many hamartomas, being feathery and pliable, could be seen only when they were floated in water.

In these 50 cases, 22 fibro-elastic hamartomas were found in 19 hearts. Although the youngest patient was 20 years old, the majority were in the seventh and eighth decades. All of the hamartomas were present in the aortic valve: one on the left coronary cusp, 14 on the non-coronary cusp, and 7 on the right coronary cusp. Several months before the start of this study, 5 hamartomas were found in a single case: 3 on the aortic valve and 2 on the chordae tendineae of the aortic leaflet of the mitral valve (Fig. 1). In this group of 50 cases, 26 aortic and 9 pulmonary valves showed fenestrations of one or more cusps. There was no correlation, however, between the presence of fenestrations and of hamartomas.

Fibro-elastic hamartomas vary from single pliable projections (Fig. 3) to large feathery papillations (Figs. 2 and 4). They occur principally on or near the free edge of the valve cusp, although occasionally they are associated with the corpus arantii. They vary from 0.3 to 1.8 cm. in length and from 0.1 to 0.5 cm. in diameter and are composed primarily of collagenous and elastic fibrous tissue (Figs. 5 and 6). From the embryologic origin and development of the valves and endo-

cardium, there is nothing to suggest either the etiology of these hamartomas or their predilection for the aortic valve, particularly its non-coronary cusp.

None of the cases reported here had specific murmurs or evidence of valvular dysfunction which could be associated with the presence of hamartomas. Because of their prominent position in the outflow tract, it would be reasonable to expect some cases to show thrombus formation or bacterial implantation. However, no clinical or anatomical evidence of such previous disease was found in any case. A hamartoma was associated with a valve showing rheumatic involvement in 3 cases.

Encountered in this study was a small hemangioma of the tricuspid valve (Fig. 7). Only 5 of these have been reported previously.[1]

SUMMARY AND CONCLUSIONS

Twenty-two fibro-elastic hamartomas on 19 aortic valves were found in 50 consecutive necropsies.

The indicated incidence is considerably greater than previously reported.

No evidence of valvular dysfunction or of bacterial implantation was associated with occurrence of these hamartomas.

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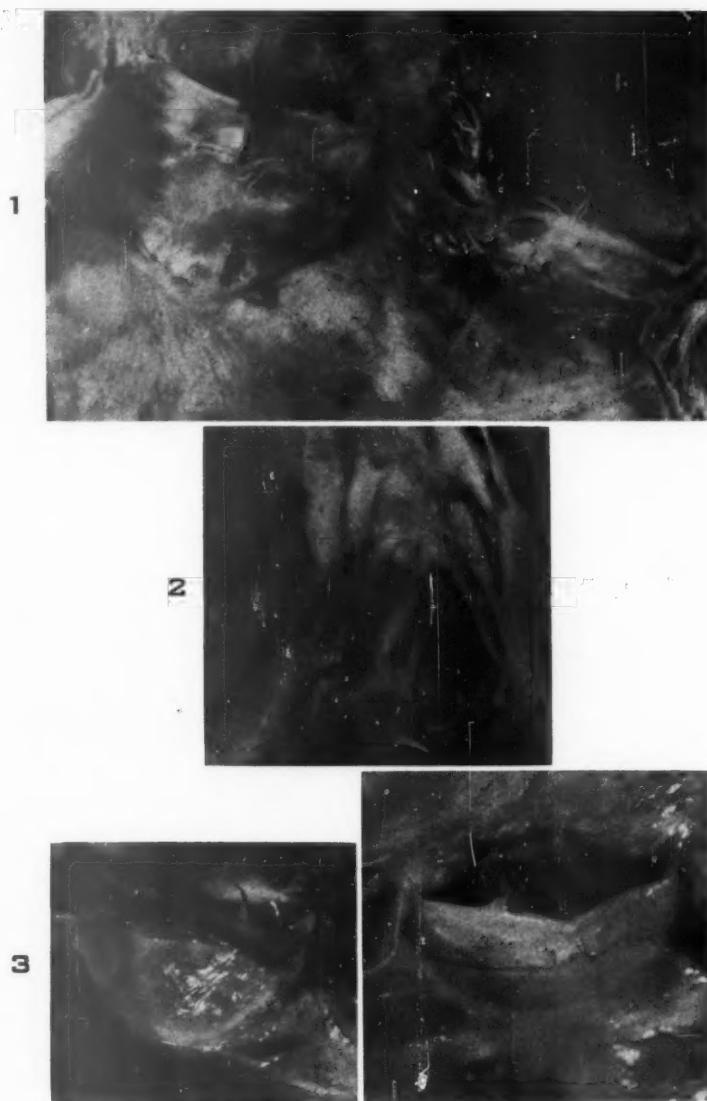
LEGENDS FOR FIGURES

Photographs for Figures 1 to 4 were taken with the hearts under water.

FIGS. 1, 3, and 4. Single and multiple hamartomas on aortic valves.

FIG. 2. Single feathery hamartoma on the chordae tendineae of the mitral valve (arrow).

Figs. 5 and 6. Fibro-elastic hamartomas. The black-staining elastic tissue component is abundant. Verhoeff's elastic tissue stain. × 15.

Fig. 7. Hemangioma of the tricuspid valve. Periodic acid-Schiff's stain. × 125.
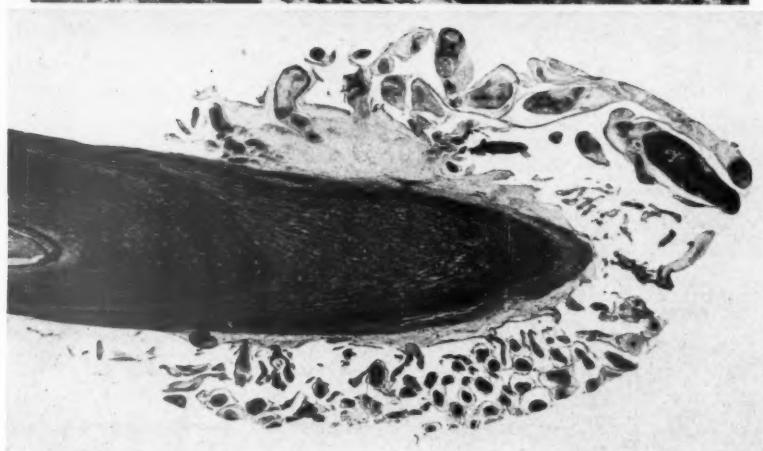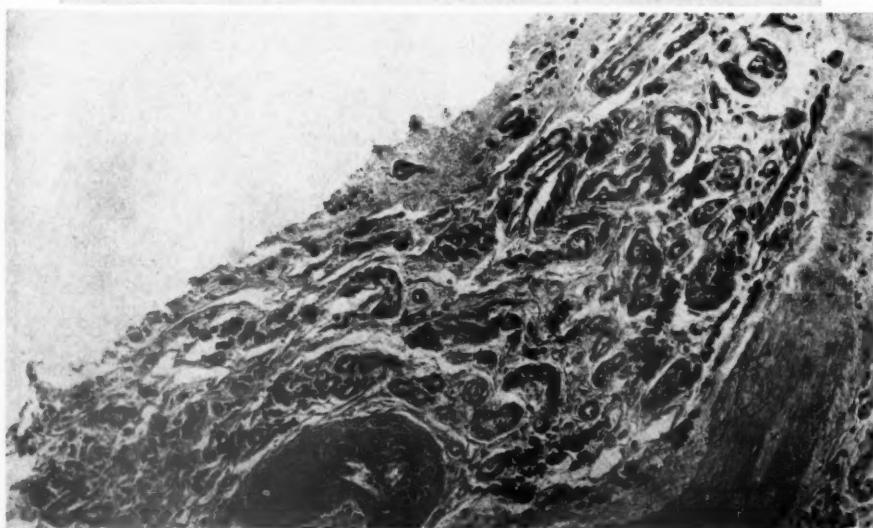
LIPOMAS OF THE THYMUS GLAND
With an Illustrative Case Report *

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Although reports on malignant tumors of the thymus gland appear fairly frequently, those on benign tumors of thymic origin are relatively rare. Perhaps the most common benign neoplasm of the thymus is that commonly referred to as a thymoma, while others are adenomas, fibromas, lipomas, and myxomas. Reference to lipomas of the thymus gland rarely is made in textbooks of pathology, and in a review of the literature only 8 verified reports of lipomas of thymic origin could be found.

The oldest recorded cases are those reported by Schmincke,[1] in 1926. He mentioned lipomas of the thymus gland which were described by Hennig and Münchmayer[2] but stated that their cases might not be true tumors since the original observations were very old.

Schmincke[1] also referred to the report of Lange[3] who, in 1904, described a tumor originating in the right lobe of the thymus gland in a woman, 58 years old. The tumor weighed 16,000 gm. Macroscopically it was lobulated, and microscopically it was made up of lobules of fat of varying size separated by connective tissue septa. Only in the area of transition between the tumor and the involved lobe of the thymus gland could thymic tissue be found.

The reviews of Bell[4] (1917) and of Margolis[5] (1931) record most of the thymic tumors reported in the older literature, but neither includes a lipoma of thymic origin. Margolis, however, stated that lipomas have been reported occasionally, but his bibliography gives no references which can be checked. Geschickter[6] (1934), in a comprehensive review of lipomas, did not describe one of the thymus gland. In the more recent literature, Schanher and Hodge,[7] Williams,[8] Bariéty and Coury,[9] Santy, Bérard, and Galy,[10] Bigelow and Ehler,[11] Gross,[12] and Rubin and Mishkin[13] have reported cases of thymic lipomas which were composed of an admixture of thymic and fatty elements. These tumors varied in weight from 170 to 750 gm. and they were all successfully removed at operation. In no instance was there evidence of malignant change within the tumors and the patients showed no symptoms of myasthenia gravis.

Bigelow and Ehler[11] referred to a case report by Andrus and Foot[14] in which a tumor weighing 2,235 gm. was removed surgically from a

child. They drew a direct comparison between this tumor and the one which they themselves reported. However, a review of the case described by Andrus and Foot reveals that in some areas of their tumor there were islands of small cells which were actively growing, with the presence of many mitotic figures, and they stated that this was the only evidence of malignant change. They diagnosed the tumor as an essentially non-malignant thymoma with rare anaplastic areas. The latter diagnosis and the histologic features which they have described leave us in some doubt as to whether other areas of this tumor, had it been completely sectioned, might not have revealed further areas of anaplasia and malignancy. We therefore do not agree with Bigelow and Ehler that this case of Andrus and Foot should be considered comparable to the tumor which the former authors described.

A review of the intrathoracic lipomas, other than those described as of thymic origin, was undertaken to ascertain if any of those recorded showed remnants of thymic tissue.[15-34] However, in none of the approximately 60 cases reported was any mention made of thymic remnants. Despite this, Rubin and Mishkin[13] stated that thymic remnants were present in the pedicle of the case reported by Beatson.[25] However, a personal review of Beatson's case showed that no microscopic study had been made of this tumor. Therefore, if we include the cases of Williams,[8] Gross,[12] and Santy, Bérard, and Galy,[10] which have been commented upon in the literature but never reported as complete cases, there are only 8 verified reports of a lipoma of thymic origin prior to this case.

REPORT OF CASE

C. F. S., a white male dental technician, 47 years old, was admitted to the hospital complaining of shortness of breath, fatigue, and swelling of the ankles. He stated that his shortness of breath dated back to the age of 3, when he suffered a crushing injury to the chest. Since that time he had been unable to undergo any strenuous physical exertion without associated respiratory distress. However, he had been able to carry on with mild exertion until 2 months prior to admission when he contracted a "chest cold." Following this, he had to sleep in a sitting position or lie flat on his stomach in order to get his breath. Despite this respiratory embarrassment, he continued with his daily work until a few days prior to admission when he noticed swelling of his feet. Inquiry revealed that the patient had a morning cough productive of mucoid sputum. He denied hemoptysis, chest pain, and chills. There were no symptoms suggestive of myasthenia gravis.

At the age of 12 he had been in a hospital with pleurisy but had had no other hospital admissions. The family history was non-contributory.

Physical examination showed a thin, slightly cyanotic, white male who was extremely short of breath. His admission temperature was 100° F. The radial pulse was regular at 160 per minute and the respiratory rate, 36 per minute. Blood pressure was 150/105 mg. of Hg. A marked venous engorgement of the head and neck was noted and the chest showed prominence of the sternum, most marked in the region of the xiphoid process. Chest expansion was poor but bilaterally symmetric. There was dullness to percussion over the entire left chest anteriorly and at both apices

posteriorly. Tactile fremitus was absent in the same areas and moist râles were heard in the region of both lung bases posteriorly. The heart sounds were heard best under the right nipple at a rate of 160 per minute, but no heart sounds could be heard in the left chest. There were no cardiac murmurs. The liver edge was palpable 10 cm. below the tip of the right ninth costal cartilage and there was pitting edema of the extremities. The remainder of the physical findings were negative.

Investigations consisted of fluoroscopy of the chest and an electrocardiogram. Fluoroscopy showed a shifting of the mediastinum to the right with generalized opaque densities of both lung fields. The electrocardiogram revealed an auricular or sinus tachycardia with a ventricular rate of 170 per minute. The patient died on the night of admission before further studies could be performed.

Necropsy Findings

When the abdomen was opened at necropsy, the liver, although not apparently enlarged, lay completely below the right costal margin. The domes of the diaphragm were convex rather than concave, and all of the abdominal organs were pushed toward the pelvis.

On opening the thorax, a large, firm mass filled the anterior compartment of the chest and completely obscured the heart and lungs (Fig. 1). It was yellowish orange and measured 34 by 30 by 10 cm. This mass was completely encapsulated and, except for an area in the region of the superior vena cava, was free of attachment to surrounding structures. On the left side there was a prolongation of the tumor extending up into the neck, anterior to the left common carotid artery. When the tumor was raised, both lungs were seen to be compressed and displaced superiorly and posteriorly. The left pleural cavity contained 300 cc. of a serosanguineous fluid, while the right side was free from fluid. There were no pleural adhesions. The heart was shifted slightly to the right and the pericardial sac was not remarkable.

The tumor weighed 6,000 gm. Serial sectioning of the mass showed that it was fairly uniformly yellowish orange and firm. The cut surface (Fig. 2) revealed a moderately thick fibrous connective tissue capsule with finer fibrous trabeculae coursing inward, giving the tumor a coarsely lobulated appearance. In the fibrous trabeculae there were large and small blood vessels.

The remainder of the gross necropsy findings were unremarkable, and a careful search yielded no other sites of tumor involvement.

Portions of the tumor were fixed in 10 per cent formalin and numerous representative sections were taken for microscopic study. These sections were stained with hematoxylin and eosin, hematoxylin-phloxine-saffron, Foot's reticulum stain, Scharlach R stain for fat, and Mayer's mucicarmine stain.

Routine sections of all other organs were taken also and stained with hematoxylin and eosin.

Microscopic study of the tumor disclosed a thick collagenous con-

nective tissue capsule (Fig. 3). The tumor parenchyma was composed mainly of mature adipose tissue supported by a scant, loose, fibrous connective tissue. Among the fatty elements in almost all sections of the tumor there were small islands of tissue readily recognizable as thymus gland (Figs. 4 to 6). These islands showed a definite admixture or infiltration with fat cells and were separated by adipose tissue. The thymic tissue was arranged in clumps and cords of small, round cells with deeply staining nuclei and a scant basophilic cytoplasm, which were identified as thymic lymphocytes. Although typical Hassall's corpuscles could be seen in only a few of the sections (Fig. 5), most areas showed small, round, calcified bodies immediately surrounded by concentrically lamellated and degenerated epithelial cells, characteristic of atrophic Hassall's corpuscles. The special staining procedures failed to reveal any further significant features, except for Scharlach R which verified the main component of the tumor as fat.

There was no evidence of malignant change in any of the numerous sections examined.

The general microscopic picture, therefore, was that of a benign encapsulated tumor composed predominantly of proliferating adipose tissue with small isolated islands of thymic tissue scattered throughout.

Routine sections from the lungs showed atelectasis together with marked congestion of alveolar capillaries and some extravasation of red blood cells into the alveoli. The liver sections showed a minimal centrilobular atrophy and congestion, with edema of the spaces of Disse. Other microscopic findings were not remarkable.

DISCUSSION

The more recent literature contains no report of an intrathoracic neoplasm of thymic origin approaching the size (6,000 gm.) of the tumor which we have described, although in the older literature the tumor recorded by Lange[3] must take precedence over all. Intrathoracic lipomas not arising in the thymus gland are reported as varying from the size of a walnut to a tumor weighing 17.5 lbs. reported by Leopold.[22] This case, therefore, represents the second largest intrathoracic neoplasm of thymic origin described in the literature.

It is also of interest to note that despite such a massive growth and the concomitant pathologic findings at necropsy, this patient complained of incapacitating symptoms for only a short period. In all of the cases previously recorded, the patients either consulted their physician because of respiratory difficulties, or the tumor was discovered on routine roentgenographic examination of the chest in time for successful surgical removal. The clinical history of the patient de-

scribed by Lange[8] could not be obtained, but the chronologic features
in his and the case recorded here are comparable, and in both cases the
true nature of the tumor was discovered at necropsy. It has been
stated[21] that the slow growth and soft consistency of intrathoracic
lipomas make it possible for some of these tumors to attain a rather
large size without causing alarming symptoms. However, it is almost
incredible to find a tumor of the size which we have described in a
man whose symptoms caused him to consult his physician only 2
months prior to death.

The possible histogenesis of this tumor composed of an admixture
of thymic and fatty elements needs explanation. Bariéty and Coury[9]
believed that most mediastinal lipomas were congenital but admitted
that some seemed to be acquired. These same authors attempted to
correlate the presence of these lipomatous growths with obesity and
the storage of fat in depots. On this basis, they proposed that tumors
of the type under discussion originate as primary neoplasms of thymic
elements which secondarily are infiltrated by adipose tissue and act as
depots for the storage of fat. The possibility of this genesis, in our
opinion, appears remote.

Involution of the thymus gland normally begins at about 4 years of
age, with a gradual thinning out of the cells of the cortex and of the
epithelial reticular cells. The area previously occupied by them is
replaced gradually by adipose tissue which is believed to arise in the
interlobular connective tissue. Hassall's corpuscles are the last ele-
ments to be replaced, and even in the aged there are scattered Hassall's
bodies surrounded by a few reticular cells and lymphocytes.[35] Lipomas
of the thymus gland, therefore, must have their origin in the adipose
tissue of the involuting gland, but the presence of an admixture of
thymic and fatty tissue must be explained.

If it were possible to show that the thymic glandular remnants as
well as the fatty tissues were proliferating, the task would be simpler.
However, in none of the numerous sections of the tumor removed at
necropsy, was there any evidence of proliferation of thymic lympho-
cytes and/or reticular cells. Almost all of the Hassall's bodies were
calcified and atrophic. Only after a prolonged search was it possible
to find a typical Hassall's corpuscle as illustrated in Figure 5.

However, despite the predominantly fatty nature of the tumor which
we have described, the over-all amount of the glandular elements was in
excess of what might be expected in a 47-year-old individual. Boyd,[36]
in her monograph on *The Weight of the Thymus Gland in Health
and in Disease,* cited the work of Hammar[37] in determining the age
changes in the histologic structure of the thymus and in determining

also the weights of the medulla, cortex, and connective tissue components. From Hammar's graphs the combined weight of the cortex and medulla from the age of 40 years onward is almost negligible. Although it was not possible to carry out a separation of elements, as was done by Hammar, it is our opinion that the weight of glandular remnants in this tumor mass of 6,000 gm. would constitute more than the normally expected 1 to 2 gm.

Therefore, we entertain two possible theories as to the histogenesis of this tumor. It is possible that under the influence of some unknown stimulus, a lipoma of multicentric origin arose which in the process of proliferation carried with it the atrophic glandular remnants of thymic tissue. It is also possible that all elements of the gland for a time showed proliferative tendencies and then at some interval the glandular elements ceased to grow, while the adipose tissue continued to proliferate. The latter theory is considered more applicable to this tumor.

How does a tumor of this type fit into a classification of tumors of the thymus gland? Bariéty and Coury[9] were the first, as far as we are aware, to use the term lipothymoma in referring to a tumor of this type, and approximately 2 years later Bigelow and Ehler[11] introduced the same name into the English literature. This new term of lipothymoma apparently has been accepted without question by Rubin and Mishkin,[13] but in our opinion it warrants some discussion.

Ewing,[38] as early as 1916, reporting on *The Thymus and Its Tumors*, began his paper thus:

No group of tumors has more successfully resisted attempts at interpretation and classification than those of the thymus. The problems involved include those which have complicated the embryological and histological study of the gland, and added difficulties arise from the comparative rarity and considerable diversity of the tumors and from the somewhat imperfect knowledge of the general pathology of the thymus.

It would be untrue to say that such a statement is still completely tenable. However, the advances in our knowledge of the thymus gland still leave many unsolved mysteries, and tumors of this gland still receive a wide and confusing variety of classifications.

As previously stated, it is our opinion that this is primarily a tumor arising from the adipose tissue of the involuting gland. It is true that the smaller tumors of this type have a greater preponderance of thymic tissue than was found in our case. However, the present case indicates that this tumor, when allowed to express its natural course, continues to grow as a lipoma while the glandular elements lose their proliferative tendency. Under these circumstances it would seem inadvisable to perpetuate the use of this new name of lipothymoma. Furthermore, the value gained from introducing this new term into an already con-

fused classification appears negligible, when an adherence to a more simple terminology is possible. We would propose, therefore, that tumors of this type be designated simply as lipomas of the thymus gland.

SUMMARY

Lipomas of the thymus gland were reviewed, together with a discussion of their nomenclature and possible histogenesis. An illustrative case report of a lipoma of thymic origin weighing 6,000 gm. and occurring in a 47-year-old white male has been included. The neoplasm was composed predominantly of fat in which remnants of the thymus persisted. It was not associated with the clinical syndrome of myasthenia gravis. It is interesting because of its immense size, the rarity of this type of tumor, and the brevity of associated clinical symptoms.

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LEGENDS FOR FIGURES

FIG. 1. The tumor *in situ*. Of note is its complete encapsulation.

FIG. 2. The cut surface of the tumor with its thick connective tissue capsule and
coarsely lobulated structure.
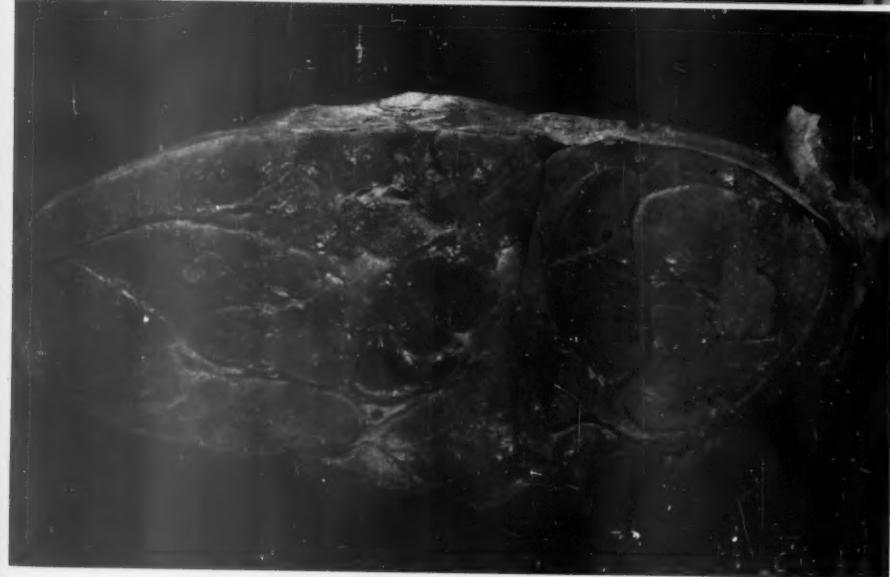
FIG. 3. The connective tissue capsule of the tumor enclosing a predominantly fatty tumor parenchyma. Hematoxylin and eosin stain. $\times$ 28.

FIG. 4. An area of tumor showing an island of thymic tissue lying in the fatty parenchyma. Hematoxylin and eosin stain. $\times$ 28.

FIG. 5. One of the rather rare typical Hassall's corpuscles with surrounding reticulum cells and lymphocytes. Hematoxylin and eosin stain. $\times$ 231.

FIG. 6. Calcified atrophic Hassall's corpuscle lying among cords of thymic lymphocytes and admixed with adipose tissue. Hematoxylin and eosin stain. $\times$ 156.
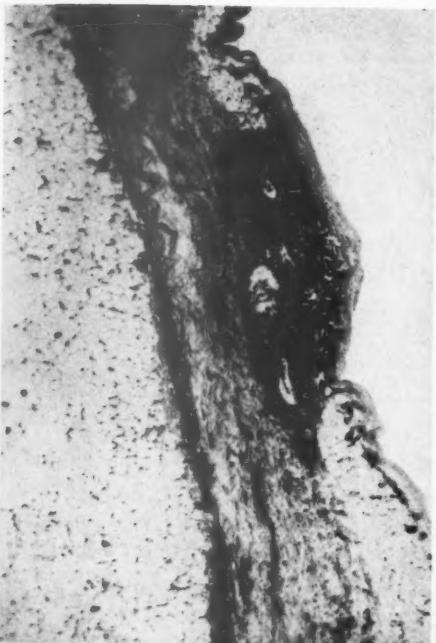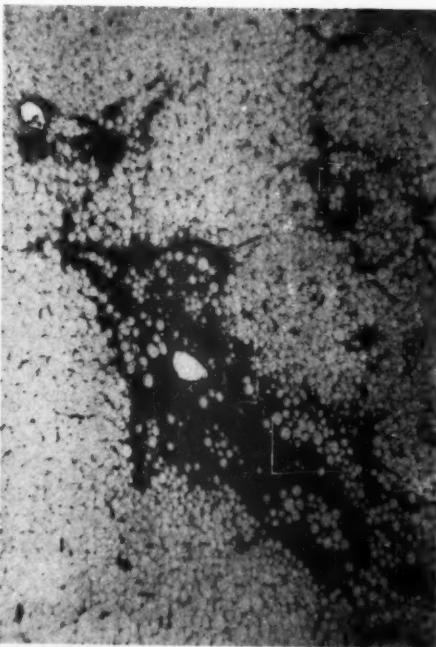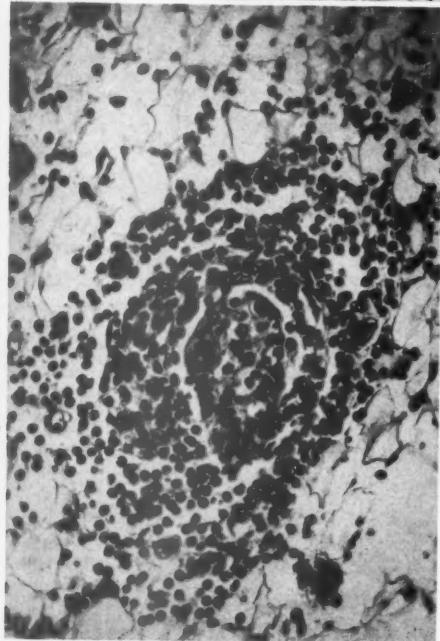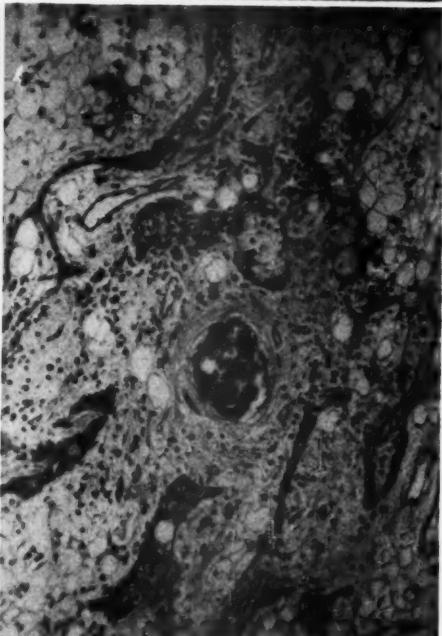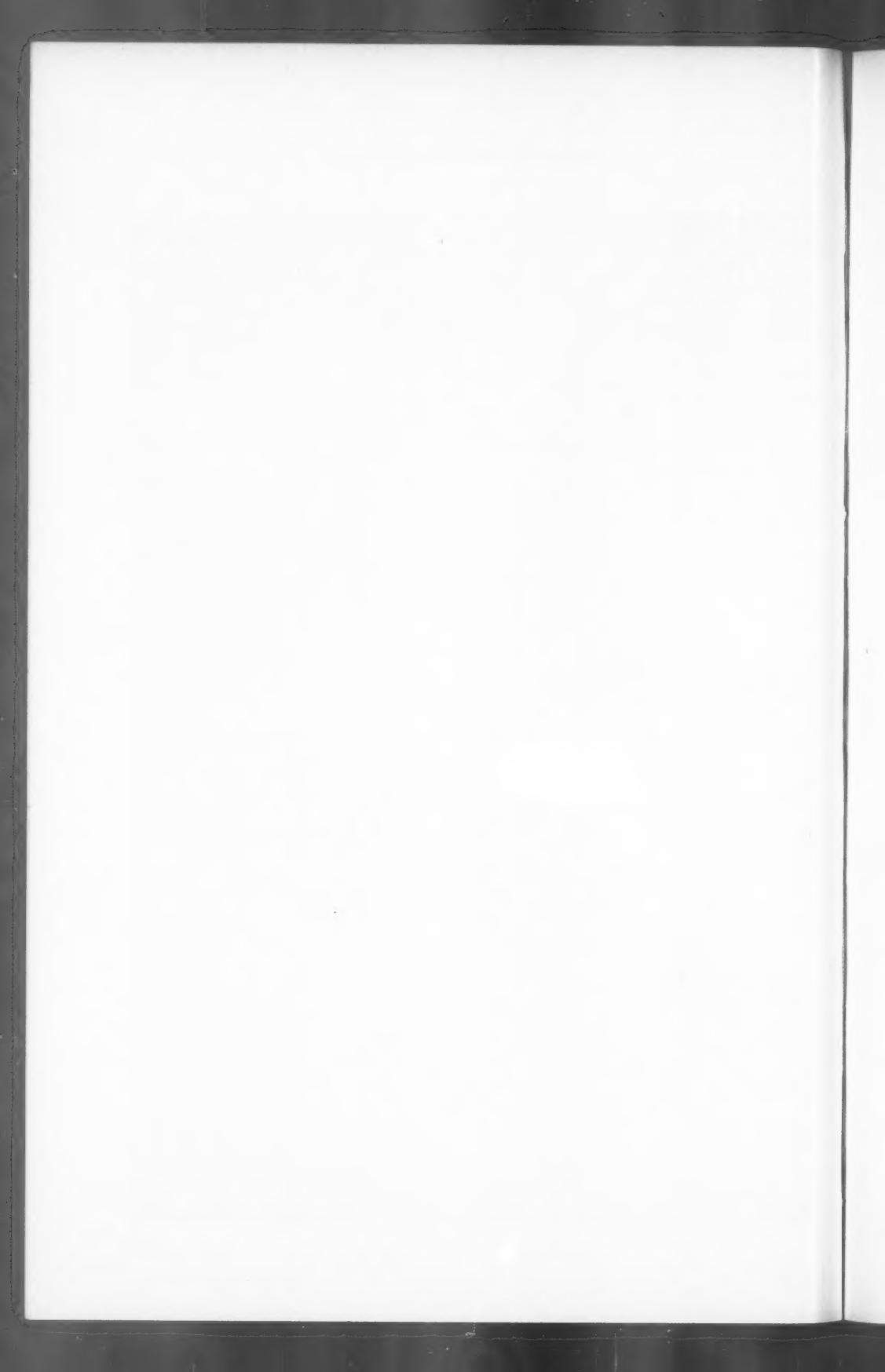
METASTASIZING INTRACRANIAL EPENDYMOMA *

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Traditionally, gliomas have been considered as a unique group of tumors which, even when highly anaplastic, almost always have their growth restricted to the confines of the central nervous system. Their most common spread is invasion into the subarachnoid space with localized meningeal involvement.[1] Also common is meningeal implantation via the cerebrospinal fluid ("meningeal gliomatosis"). Thus, Cairns and Russell[2] found 8 examples of such meningeal seeding in a series of 22 consecutive necropsies of patients with glioma. Similarly, in a review at the Mayo Clinic, Polmeteer and Kernohan[3] found 42 examples of such seeding. A distinctly less common type of spread is direct extension into the overlying skull and scalp; several examples were cited by Maass.[4]

The full malignant potentialities of gliomas are realized when spread occurs to distant organs, in a manner similar to the metastasis of other tumors, but such cases are rare. Maass[4] listed only 13 examples in his review of the subject and considered most of them to be of questionable validity. The literature records other reports of intracranial tumors with extracranial metastases, but these include non-gliomatous tumors such as meningeal sarcomas, meningiomas, pineal tumors, hemangioblastomas, melanomas, and pituitary tumors, and will not be discussed further in this paper.

Ependymomas spread in a fashion similar to other gliomas. Thus, 5 of the 42 cases of meningeal gliomatosis reviewed by Polmeteer and Kernohan[3] involved ependymomas. Direct extension into the skull was illustrated by 2 cases reported in 1951. In one of these,[5] ependymoblastoma had penetrated dura and eroded the craniotomy bone flap. In the other case,[6] a "malignant ependymoma" had "penetrated through a decompression area in the skull and around the margins of a bone flap to form a large bulky mass beneath the scalp, infiltrating the fibrous connective tissue of the scalp and the corium of the skin." Hicks and Warren[7] illustrated another example of invasion of the skull and scalp by ependymoma.

Cases of ependymoma with distant metastases are exceedingly rare. We have found only 2 authenticated reports, both of which appeared

in recent literature. One of these was confirmed by necropsy,[8] the other by lymph node biopsy.[4] Ours is another necropsied case. These 3 cases illustrate that ependymomas occasionally can behave like any other cancerous neoplasm.

To assist in the appraisal of the rare cases of metastasizing tumor of the central nervous system, Weiss[8] recently has outlined four criteria which should be satisfied before a report is accepted: (1) The presence of a single, histologically characteristic tumor of the central nervous system must have been proved. (2) The clinical history must indicate that initial symptoms were due to this tumor. (3) A complete necropsy must have been performed and reported in sufficient detail to rule out the possibility of any other primary site. (4) The morphologic appearance of the tumor of the central nervous system and of the distant metastases must have been identical, with due allowance for differences in degree of anaplasia.

Maass's case[4] was that of a 27-year-old male who was admitted to a hospital in 1951 with neurologic symptoms of 8 months' duration. Craniotomy revealed a tumor, 5 cm. in diameter, invading the left occipital cortex. A histopathologic diagnosis of ependymoblastoma was reported by the Armed Forces Institute of Pathology. In 1952, the patient was readmitted with severe neurologic symptoms. The operative site was reopened and a large intracerebral cyst and several tumor fragments were removed. Microscopic examination revealed a typical ependymoma pattern with pseudo-rosette formation. X-ray therapy was given before discharge. The patient was readmitted in 1953 with symptoms of increased intracranial pressure. Six months previously, hard masses had developed in his neck. Examination revealed a mass, 4 by 6 cm., occupying the left lateral cervical area, and on the right side a small lymph node, measuring 1 by 2 cm., was palpable. Roentgenograms of the chest were normal. Removal of the right cervical node showed a tumor which was very similar to the tumor removed at craniotomy in 1952. Re-exploration of the skull revealed considerable necrotic cortex extruding through previous burr openings. A left occipital lobectomy was performed. Microscopic examination showed only necrotic and edematous tissue. Death occurred 2 months later. Permission for necropsy was refused.

Even though metastases were proved by lymph node biopsy, Maass[4] placed his own case in a "questionably valid" category because confirmation by necropsy was lacking.

More recently, a case of metastasizing ependymoma of the cauda

equina was reported by Weiss.[8] The patient, a 22-year-old male, was admitted to a hospital in 1943 with pain in the lower back which radiated down both legs. At operation, a large intrathecal tumor was found, displacing most of the cauda equina. Because several nerve roots were embedded, the tumor was removed piecemeal. X-ray therapy was given because it was feared that the tumor had not been removed completely. The patient remained well until 1945 when he developed symptoms of compression of the spinal cord. At operation, a tumor mass with included nerve roots was removed, following which the patient became completely paraplegic. A third operation was performed in 1946, at which time the tumor was believed to have invaded vertebrae and intervertebral spaces. Another operation was performed in 1950; the tumor extended through the dura and appeared to have invaded the bone. Complete removal was not possible. In 1951, needle biopsy was carried out on a paraspinal tumor mass. The patient died in 1953. Necropsy was performed.

Histologic sections of all surgical specimens and of the tissue obtained for biopsy showed the structure of ependymoma of myxopapillary type. A similar structure was present in the tumor removed at necropsy. The neoplasm had replaced the lower vertebral column almost completely and extended anteriorly to form a large retroperitoneal mass. The tumor was present within the common iliac veins and inferior vena cava. Metastatic deposits were found in the liver, lungs, small pulmonary arteries, tracheobronchial lymph nodes, and beneath the parietal pleura.

Weiss[8] stated that his was the first reported case of an ependymoma with metastases to distant organs. Our case completely fulfills his criteria and thus, to our knowledge, is the first necropsied case of *intracranial* ependymoma with distant metastases.

REPORT OF CASE

M. R., a 16-year-old white female, was admitted to the University of Alberta Hospital on November 26, 1941, complaining of severe generalized headaches of 3 months' duration, vomiting for 3 weeks, and internal strabismus of one eye for 10 days.

Physical examination revealed a well nourished female with marked internal strabismus of the right eye and bilateral papilledema. Ventriculography suggested the presence of a large tumor in the left parietal region. At craniotomy, the dura was under considerable tension, and, on reflecting it, a tumor was seen, presenting in the left parietal region about half way between the base of the brain and the median fissure and coming to the surface about 2 cm. behind the post-central sulcus. The tumor was excised.

The pathologic specimen consisted of an irregular, nodular mass of coarse, friable,

gray neoplastic tissue with a thin overlying layer of cortex. It measured 6 by 5 by 4 cm. Normal brain tissue could not be recognized on the deep surface of the specimen. Microscopic sections showed the typical structure of a grade 1 ependymoma (Fig. 2) with a predominant pseudo-rosette pattern.

The patient's postoperative course was uneventful, and she remained well, subsequently married, and delivered one normal child.

She was readmitted to the University Hospital 7 years later, on May 11, 1948, having developed neurologic symptoms of increasing severity during the previous 7 months. A space-occupying lesion was diagnosed, and at craniotomy a large tumor was found in the left occipital area. This extended forward into the posterior half of the parietal region. Although the tumor was attached to the falx cerebri and the dura in the midline, it was stripped off by blunt dissection without injury to the sagittal sinus.

The pathologic specimen on this occasion consisted of an irregular, coarsely nodular tumor mass which measured 8 by 6 by 5 cm. and contained a number of small cysts filled with brown fluid. Microscopic sections again showed the structure of grade 1 ependymoma.

The patient was discharged on the 13th postoperative day.

Three years later, she suddenly developed severe symptoms of increased intracranial pressure and was readmitted to the hospital on April 15, 1951, when it was believed that another craniotomy was imperative. At operation, the posterior left cerebral hemisphere was replaced, for the most part, by solid, gray neoplastic tissue which was very nodular and irregular. The tumor mass, the remaining portion of occipital lobe, and much of the left parietal lobe were excised. Some neoplastic tissue was left near the brain stem because the patient's condition did not warrant further operative trauma. The operation was followed by a course of x-ray therapy, and the patient was discharged on the 15th postoperative day, much improved and walking.

The pathologic specimen again showed the typical structure of grade 1 ependymoma, large portions of which were necrotic.

During the following 3 years, she was readmitted to the hospital on six different occasions because of epileptiform seizures, increasing weakness of the right arm and leg, loss of vision in the right eye, depression, and confusion. Treatment consisted largely of anticonvulsant medications. On one admission (July, 1953), small nodules were noted on the left side of the scalp, which were "itchy and tender," but these were not described in further detail.

The patient's final admission was on August 14, 1954, at the age of 29 years. She was semi-stuporous and pursued a steadily downhill course until death on September 3, 1954, 13 years after the initial onset of symptoms and the first craniotomy.

NECROPSY FINDINGS

Necropsy was performed 15 hours post mortem.

The body was emaciated, measuring 63 in. in length and weighing about 90 lbs. The external appearance was essentially unremarkable.

Lungs. The left lung weighed 420 gm. and the right, 400 gm. The surfaces of both were studded by many slightly umbilicated, very firm, yellowish gray nodules which ranged from 1 to 5 mm. in diameter. Multiple cross sections revealed such nodules to be present also deep within the parenchyma of all lobes (Fig. 6).

Lymph Nodes. Cervical, mediastinal, and abdominal lymph nodes were not enlarged and were of uniform fleshy consistency.

Heart. The heart weighed 220 gm. and showed no gross abnormality.

Spleen, Kidneys, and Liver. The spleen, kidneys, and liver showed only a moderate degree of passive congestion.

Head. On the left side of the scalp, along the old incisional scars, there were three firm, pinkish white, subcutaneous nodules which measured 0.5, 0.7, and 1.0 cm. in diameter and extended from the corium to the galea. Reflection of the scalp revealed an operative bone flap held in place by fibrous tissue. The calvarium was removed with difficulty because the dura was firmly adherent by scar tissue in the left parieto-occipital region. There was no invasion of the skull by tumor. Beneath the dura, numerous yellowish gray tumor nodules, ranging in diameter from 0.4 to 1.4 cm., compressed the sagittal, left lateral, and left straight sinuses, but there was no apparent luminal invasion.

The brain, with attached dura, weighed 1,280 gm. The right cerebral hemisphere was unremarkable. On the left, a coarsely lobular tumor mass, measuring 8 cm. in length and 5 cm. in diameter, occupied the position of the previously excised left occipital lobe and most of the left parietal lobe. Multiple horizontal cross cuts showed the tumor to be formed of several large, irregularly lobular masses which averaged 4 cm. in diameter. Some of these masses contained multiloculated cystic spaces filled with gelatinous material (Fig. 1). The tumor was separated from the wall of the left lateral ventricle by brain substance which was compressed to a thickness of only 2 mm.

Microscopic Findings

Sections were stained with hematoxylin and eosin, iron hematoxylin, and Mallory's phosphotungstic acid hematoxylin.

A review of the sections of tumor removed at previous craniotomies showed features similar to those found in the sections of necropsy material. All exhibited the typical features of grade 1 ependymoma.

Brain Tumor and Dura. Sections of the brain tumor and dura showed a predominantly pseudo-rosette pattern, with mature columnar ependymal cells possessing faintly fibrillar processes orientated radially around a central acidophilic body (Fig. 3). There was no anaplasia, hyperchromatism, or mitotic activity. Frequently, the tumor showed papillary formation (Fig. 8), and occasionally there were areas of glandular arrangement. Blood vessels traversing neoplastic tissue were

well formed and intact. There were scattered areas of necrosis and fibrosis in most sections. Blepharoplasts were not demonstrated in these or other sections by the staining methods employed.

Multiple sections through dural sinuses failed to reveal any break in the endothelial lining.

Scalp. Sections of the nodules of the scalp showed a predominantly glandular and papillary pattern (Fig. 7), with only a few pseudo-rosettes present. Considerable fibrous tissue surrounded the tumor elements.

Lungs. Metastatic nodules of well differentiated ependymoma were contained within pulmonary alveoli (Fig. 4). Most exhibited a pseudo-rosette pattern (Figs. 4 and 5), but some were predominantly papillary. No areas of glandular formation were present. The larger nodules contained central areas of necrosis and a slight amount of reactive fibrosis. No tumor emboli were found within vascular or lymphatic channels. There were patches of bronchopneumonia and pulmonary edema.

Other Organs. Sections of heart, spleen, kidneys, urinary bladder, uterus, ovaries, vertebral bone marrow, esophagus, stomach, large intestine, pancreas, adrenal glands, liver, gallbladder, and pituitary gland were not remarkable. Multiple sections of cervical, paratracheal, peribronchial, and para-aortic lymph nodes showed no evidence of tumor.

COMMENTS

In comparing our case of metastasizing ependymoma with the 2 cases in the literature, there would appear to be four significant features: (1) prolonged survival, (2) multiple craniotomies, (3) nodules of the scalp, and (4) multiple small pulmonary metastases.

Prolonged Survival

A common feature of all 3 cases was postoperative survival for a period of years. Our patient lived for 13 years, Weiss's[8] patient for 10 years, and Maass's[4] for 2½ years. According to Kernohan's group,[1,9] the clinical course parallels the histologic degree of malignancy of the ependymoma, the patients with grade 1 tumors surviving an average of 6.3 years, those with grade 2 tumors surviving 3.1 years, and those with grade 3 tumors living 1.5 years postoperatively. Using Kernohan's method of grading, our case would be classed as grade 1, as would Weiss's, judging from his photomicrographs. Maass's case appears more anaplastic, perhaps grade 2. Our patient and that of Weiss sur-

vived about twice as long as the average for Kernohan's grade 1 group (although we realize that Kernohan's data[9] apply to intracranial ependymomas and thus may not be strictly applicable to Weiss's case of cauda equina tumor).

Thus, while histologically well differentiated ependymoma might be expected to metastasize less readily than a more anaplastic tumor, the prolonged survival time which may occur with more benign appearing tumors seems to favor eventual dissemination outside of the central nervous system.

Multiple Craniotomies

Weiss's[8] patient underwent four operations for his cauda equina tumor, while Maass's[4] and our patient each had three craniotomies. Surgical intervention disrupts natural barriers to tumor spread, e.g., dura and fascial planes. Thus, Weiss commented that in his patient, growing residual tumor may have reached the vertebral column through gaps left in the dura following surgical intervention. Extensive tumor growth in retroperitoneal soft tissues followed, with invasion of iliac veins and subsequent multiple, distant, blood-borne metastases.

In our case, the soft tissue growth of tumor first appeared as nodules of the scalp in the surgical scars 27 months after the last craniotomy. Fourteen months later, at necropsy, the largest of these nodules measured about 1 cm. in diameter. No evidence of direct spread of tumor from brain to skull or scalp was visible at necropsy, suggesting that these lesions were the result of implantation of tumor cells during craniotomy. The interval between operation and appearance of the nodules is consistent with the latent period sometimes observed with other tumors, and may represent a period of "acclimatization" of ependymoma cells to an extracerebral environment.

Nodules of the Scalp

We noted that the histologic pattern of the nodules of the scalp showed a rather striking predominance of glandular and papillary architecture, with only a few pseudo-rosettes present, in contrast to brain and dura where the pattern was predominantly that of pseudo-rosettes. These structural differences probably are attributable to altered environment.

James and Pagel[10] also commented on this change of pattern with environment in their report of a necropsied case of metastasizing oligodendroglioma. Nodules of the scalp were present in which the tumor pattern was altered and "not unlike scirrhous carcinoma."

The work of Zimmerman[11] furnishes an experimental counterpart to these findings, namely, that soft tissue growth of gliomas results in architectural alteration. Zimmerman transplanted chemically induced ependymomas into the subcutaneous tissues of mice. Transfer of these tumors to chick allantoic membrane resulted in total loss of pattern, but when the cells were transferred back to mouse subcutis, the characteristic structure of ependymoma was reconstituted. Zimmerman therefore emphasized the important influence which environment has on the microscopic structure of glial tumors.

Pulmonary Metastases

The multiplicity of small tumor nodules scattered throughout both lungs in our case indicated a relatively recent generalized seeding. The absence of metastatic tumor in cervical and peribronchial lymph nodes, and the absence of tumor emboli in pulmonary lymphatic channels, strongly suggested that spread to the lungs did not occur by the lymphatic system, but rather by a hematogenous route. It has been suggested[12] that surgical intervention opens venous channels and that the intraluminal negative pressure favors the aspiration into their lumina of viable tumor cells which are "filtered out" in the lungs. Dissemination during surgical procedures does not seem likely in our case, since the largest pulmonary metastasis measured only 5 mm. in diameter, 41 months after the last craniotomy. A larger size would be expected if spread had occurred that long ago.

It seems more likely that tumor cells entered the dural sinuses some time later, even though we could not demonstrate such a site in relation to the multiple tumor nodules compressing these vascular channels. Zimmerman[13] has stated that gliogenous neoplasms seem unable to invade blood vessels, but Weiss's[8] finding of ependymoma within iliac veins contradicts this view.

Final Comments

Human examples of locally invasive and metastatic growth by ependymomas, as well as experimental transplantation of these tumors, indicate that they will proliferate in foreign tissues as do other cancerous neoplasms. This occurs rarely. Theories concerning the infrequency of extracranial spread of gliomas have been reviewed by other authors[4]; suggestions have included the "blood-brain barrier," the absence of lymphatic connections with intracranial structures, and the difficulty with which gliomas penetrate blood vessels.[4,13]

The case reported by us indicates that implantation of ependymal

tumor cells in the scalp may follow attempts at surgical removal of such neoplasms. Other authors[10] have suggested that secondary metastases can occur from such foci by both lymphatic and hematogenous routes.

We feel that improvements in anesthetic, surgical, and therapeutic techniques, by prolonging survival and permitting multiple operations, will increase the number of cases of metastasizing glioma. Concerning therapeutic techniques, we feel it worth while to note that in all 3 cases of metastasizing ependymoma the patients received considerable x-ray therapy. In this regard, it is of interest that Zimmerman[11] has shown experimentally that x-irradiation accelerates the rate of growth of gliomas and alters their morphologic appearance.

SUMMARY

Gliomas rarely metastasize outside the central nervous system. In the English literature, we found only one report of metastasizing ependymoma (of the cauda equina) which was confirmed by necropsy. This paper was discussed, together with another report in which metastatic spread of ependymoma to cervical lymph nodes was established by biopsy, although necropsy was not performed.

We have reported the case of a patient with a recurrent parieto-occipital ependymoma (grade 1) on whom three craniotomies were performed at long intervals, contributing to a 13-year period of survival. At necropsy, ependymomal scalp implants and multiple pulmonary metastases were found.

It is concluded that ependymomas may, on occasion, act like truly malignant neoplasms, capable of soft tissue invasion and distant metastatic spread. We feel that this rare behavior is favored by prolonged postoperative survival and, perhaps, by multiple operations. The possible unfavorable effects of x-ray therapy were mentioned.

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LEGENDS FOR FIGURES

FIG. 1. Horizontal section through the left cerebral hemisphere, showing the nodular and partly cystic tumor mass replacing occipital and parietal lobes. The overlying dura is adherent. Necropsy specimen. × ¾.

FIG. 2. Section of brain tumor showing well formed pseudo-rosettes typical of low-grade ependymoma. Operative specimen from first craniotomy in 1941. Hematoxylin and eosin stain. × 330.

FIG. 3. Cellular detail of recurrent brain tumor, 13 years after first craniotomy. The pseudo-rosette pattern is almost identical with that in Figure 2. Necropsy specimen, 1954. Hematoxylin and eosin stain. × 330.

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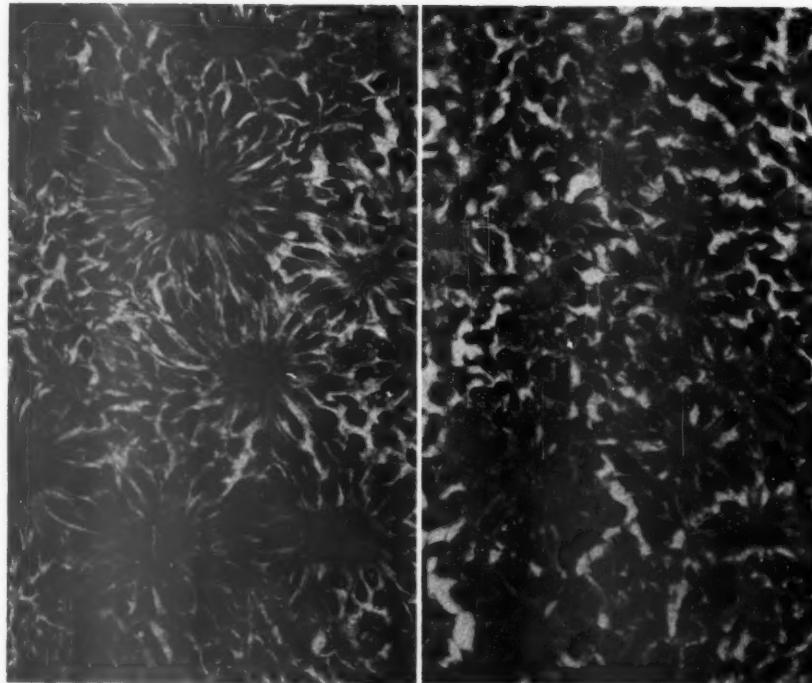
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FIG. 4. Metastatic ependymoma in pulmonary alveoli. Normal lung parenchyma is present on the left of the photograph. Hematoxylin and eosin stain. × 163.

FIG. 5. Cellular detail of metastatic ependymoma in lung, showing pseudo-rosette pattern similar to that in tumor of the brain (Fig. 3). Hematoxylin and eosin stain. × 330.

FIG. 6. Gross appearance of metastatic nodules of ependymoma within lung. × 1.5.

FIG. 7. Ependymoma growing in deep corium of scalp. Here the tumor has a predominantly glandular pattern. Hematoxylin and eosin stain. × 60.

FIG. 8. Grade 1 ependymoma with pseudo-rosette and papillary pattern invading dura at the right side of the field. Operative specimen from third craniotomy, 1951. Hematoxylin and eosin stain. × 285.
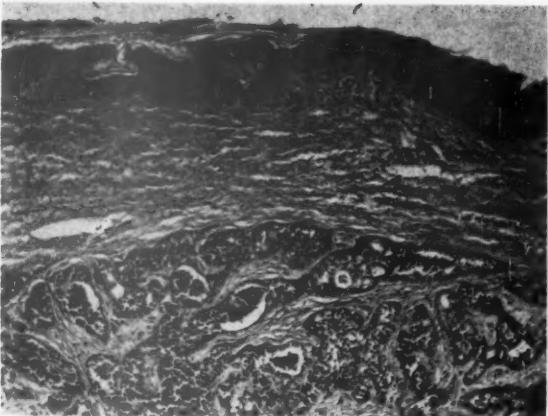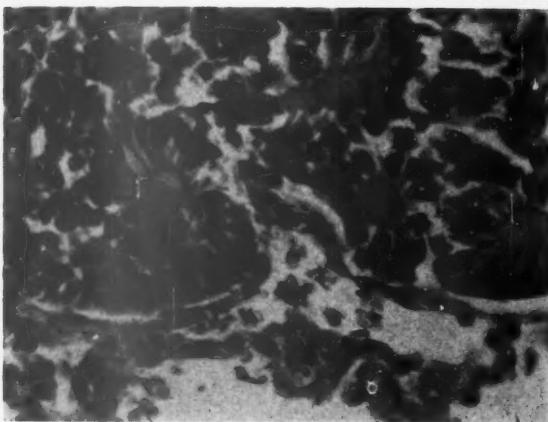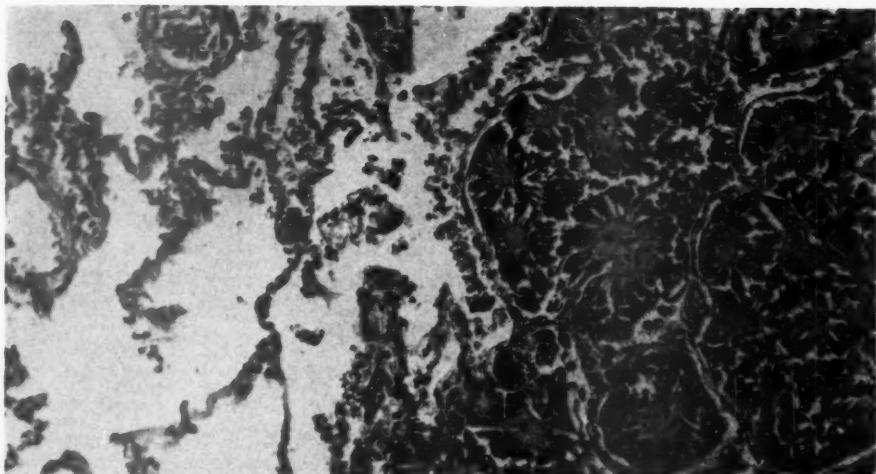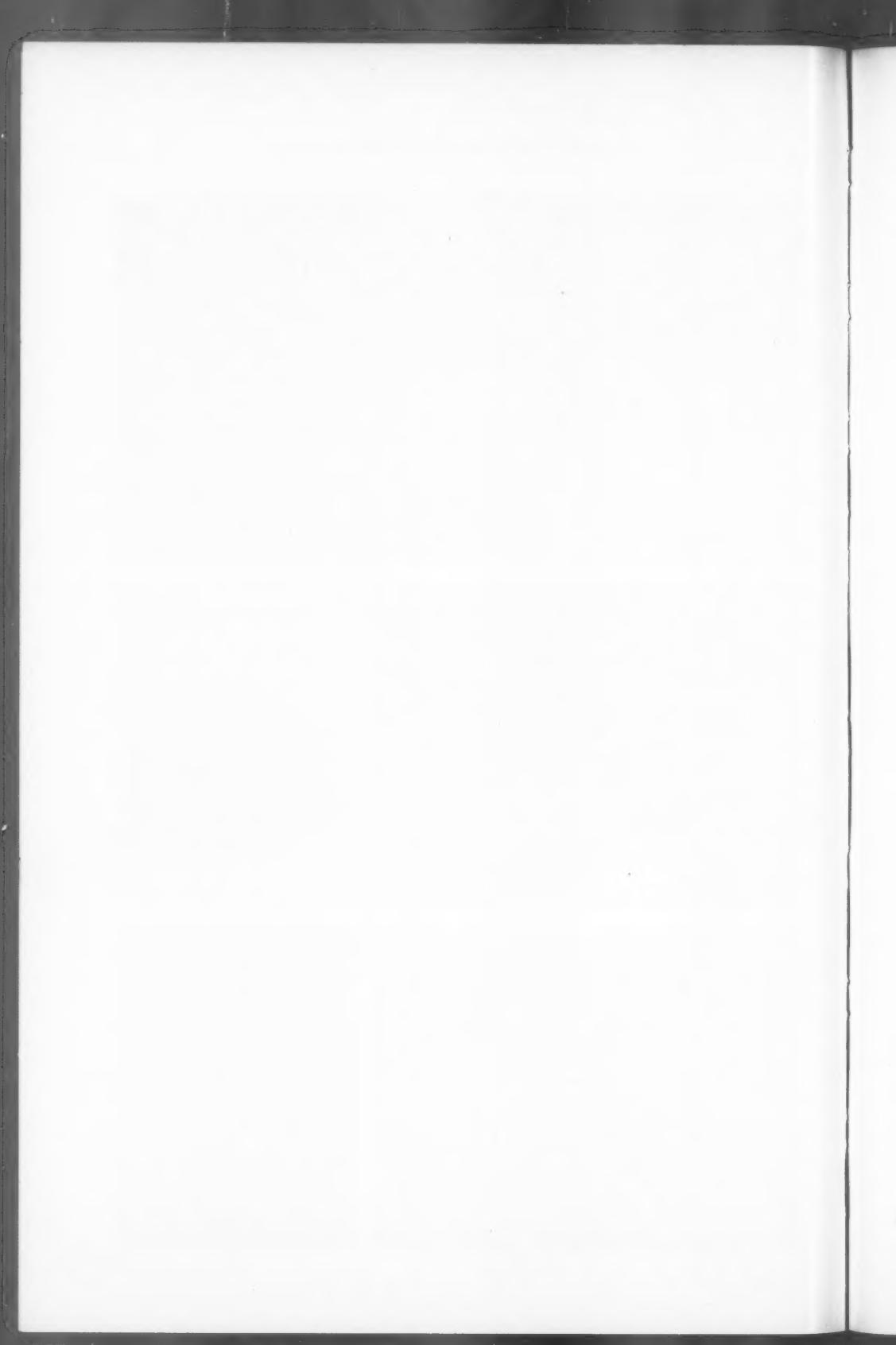
ANEURYSMS OF THE AORTA

A CLINICOPATHOLOGIC STUDY OF 369 NECROPSY CASES *

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Selected patients with aneurysms of the aorta have been shown to respond well to surgical treatment, since recent advances have permitted the surgeon to expand his operative field to include the heart and aorta. Greater diagnostic skill is required of physicians to achieve earlier diagnosis so that proper therapy may be instituted; and currently the medical profession is found to be demonstrating new interest in aortic aneurysms as evidenced by the increasing number of publications on this subject. Knowledge of such abnormalities should be based on studies of clinicopathologic correlations and of the natural biologic behavior of aneurysms. Several such articles[1-8] concerning aneurysms of the aorta have appeared within the past 100 years, and prior to that time the work of Erichsen[9] was outstanding.

A review of the literature showed the incidence of aortic aneurysms in necropsies to vary from 2.2 per cent[1] to 4.36 per cent.[10] In all series the male sex predominated; in one report the lesions were found more frequently in the white race[1] while in other series the Negro race was affected more often.[2,4,11] Patients with aortic aneurysms were commonly in the fourth, fifth, or sixth decades of life; syphilis was the chief etiologic factor, and the lesion usually was saccular.[1-4] Dissecting aneurysms, however, tended to occur in later years,[5,6] with some exceptions,[8,12] and generally were due to cystic medial necrosis.[5,6,8,12] A majority of all aneurysms were located in the ascending limb of the aortic arch.

Clinically, aneurysms of the ascending limb have been known as "aneurysms of symptoms" while those of the transverse portion of the arch have been designated "aneurysms of signs."[2,11] The outstanding symptoms were pain, dyspnea, and cough, while the main signs were dullness, tracheal tug, and a pulsating mass.[2,4,11] Some aneurysms, however, remained completely silent.[1,2,4] Dissecting aneurysms presented the most consistent clinical findings with the sudden onset of pain.[5,6,8] The average duration of illness in patients with aortic aneurysms was given in various reports as from 6 months[2] to over 2

years.[1] Death usually was ascribed to one of four means: rupture, pressure, heart failure, or some entirely unrelated cause. Rupture of saccular aneurysms occurred in one third[1] to one half[11] of the patients, and there was an even greater frequency of rupture in dissecting aneurysms.[8] Pressure upon a vital structure by the aneurysmal sac was responsible for death in 5 per cent[10] to 18 per cent[2,3] of the patients with aortic aneurysms, while heart failure accounted for 10 per cent[2] to 20 per cent[10] of the fatalities.

The following represents a clinicopathologic study of a large number of aortic aneurysms encountered at necropsy.

MATERIALS AND METHODS

The data for this publication are based on necropsies performed in the Pathology Department of the University of Texas Medical Branch at Galveston from 1892 to 1953. During this 62-year period 9,273 necropsies were recorded and in 369 cases one or more aneurysms of the aorta were demonstrated. The multiple lesions found in some aortas brought the total number of aneurysms studied to 412. Both the clinical charts and necropsy protocols were reviewed and the material for our tabulations consists only of unequivocal positive findings from these sources. Doubtful information is included in the "absent" or "not given" category. In certain instances the clinical write-ups were incomplete because the patient was unconscious, incoherent, or illiterate, or because of some other extenuating circumstance. A few clinical charts were missing.

We considered the anatomical location of the aortic aneurysms to be an important feature since the majority of the clinical findings can be related to the actual position of the lesion. One aspect of the tabulation of our data, therefore, centered about the involved segment of the aorta, and for our report the aorta was divided into five basic anatomical segments: ascending arch, transverse arch, descending arch, descending thoracic aorta, and abdominal aorta. Single aneurysms ordinarily involved only one of these anatomical segments although at times a single aneurysm was quite large and not restricted to a single segment. Since the exact location of an aneurysm usually is determined in clinical evaluation, the single lesions limited to only one anatomical segment and the single lesions involving more than one anatomical segment were treated as separate categories in our tabulations. In some aortas more than one aneurysm was demonstrated and these were handled in a special category in an attempt to find some

feature peculiar to multiple lesions. Dissecting aneurysms also were placed in a separate category since they present a distinct entity.

Our cases were accumulated over a prolonged period of time, 1892 to 1953, and by comparing the first 100 consecutive cases of aortic aneurysms with the most recent 100 cases we were able to observe certain significant changes not readily apparent when the series was dealt with as a whole. The first 100 cases of aortic aneurysm occurred between 1892 and 1928 during a time of now outmoded therapy, particularly for syphilis, and the last 100 cases occurred from 1943 to 1953 during the era of antibiotics. These evaluations are listed separately from the results of the over-all survey.

Incidence and Location

The 369 cases of aortic aneurysm in our series of 9,273 necropsies give an over-all incidence of 3.98 per cent. The majority of these lesions, 56.1 per cent, involved only one anatomical segment and most of these were within the ascending portion of the arch. Multiple aneurysms of the aorta were found in 10.6 per cent and dissecting aneurysms were encountered in 11.9 per cent. Table I gives a complete breakdown of the series according to location.

Predisposing Factors

The majority of our subjects, 62.4 per cent, were in the fifth, sixth, and seventh decades of life, although the youngest was 8 years of age and the oldest 88 years old. In 5 instances the age was not recorded. Table II lists the age groups according to the anatomical location of the aneurysm and its morphologic type. It demonstrates that, in general, aneurysms of the arch of the aorta occur in younger individuals while those of multiple or dissecting types and those of the abdominal aorta tend to occur in older patients.

Aortic aneurysms were observed most frequently in the Negro race, 61.2 per cent, although two exceptions were found when the data were evaluated with regard to the anatomical location and morphologic type. Dissecting aneurysms were almost evenly distributed between the races, 45.5 per cent being in the white race. Aneurysms limited to the abdominal aorta occurred more commonly in the white race, 67.5 per cent. The male sex, 83.7 per cent, clearly predominated in our series, and this predominance was true regardless of anatomical location or morphologic type. Concerning the patient's occupation as a possible predisposing factor to aneurysm formation, it is of interest to

TABLE I

Anatomical Location of 412 Aortic Aneurysms According to Morphologic Type (369 Cases)

Anatomical segment	Total aneurysms of all types (412 aneurysms)		Saccular and fusiform types				Multiple aneurysms		Dissecting aneurysms	
			Single aneurysm involving only one anatomical segment (207 aneurysms)		Single aneurysm involving more than one anatomical segment (79 aneurysms)		One or more anatomical segments (30 cases, 82 aneurysms)		One or more anatomical segments (44 aneurysms)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Aneurysms involving single anatomical segments										
AA	120	29.1	82	39.6			27	32.9	11	25.0
TA	47	11.4	42	20.3			5	6.1		
DA	48	11.6	31	15.0			16	19.5	1	2.3
DT	28	6.8	12	5.8			12	14.6	4	9.1
Ab	60	14.6	40	19.3			14	17.1	6	13.6
Aneurysms involving more than one segment										
AA, TA	13	3.2			10	12.7			3	6.8
AA, TA, DA	55	13.3			49	62.0	4	4.9	2	4.5
AA, TA, DA, DT	8	1.9			5	6.3	1	1.2	2	4.5
AA, TA, DA, DT, Ab	13	3.2			3	3.8			10	22.7
TA, DA	10	2.4			7	8.9	1	1.2	2	4.5
TA, DA, DT	1	0.2			1	1.3				
TA, DA, DT, Ab	2	0.5			1	1.3			2	4.5
DA, DT	1	0.2			1	1.3				
DT, Ab	6	1.5			3	3.8	2	2.4	1	2.3

AA = Ascending arch.   TA = Transverse arch.   DA = Descending arch.   DT = Descending thoracic.   Ab = Abdominal.

TABLE II

The Ages of Patients with Aortic Aneurysms According to Morphologic Type and Anatomical Location (369 Necropsies)

Age groups	Total frequency by age groups (369 cases)		Frequency by age groups of saccular and fusiform aneurysms												Dissecting aneurysms	
			Involving one anatomical segment only										Involving more than one anatomical segment		Frequency by age groups, any location (44 cases)	
			Ascending aortic arch (82 cases)		Transverse aortic arch (42 cases)		Descending aortic arch (31 cases)		Descending thoracic (12 cases)		Abdominal aorta (40 cases)		Single lesion (70 cases)		Multiple lesions (30 cases)			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Under 20	1	0.4	1	1.2														
20–29	12	3.3	4	4.9	1	2.4	1	3.2			1	2.5	2	2.5	3	7.7		
30–39	50	13.6	11	13.4	10	23.8	9	29.0			3	7.5	10	12.7	2	5.1	5	11.4
40–49	81	22.0	20	24.4	11	26.2	6	19.3	1	8.3	5	12.5	23	29.1	7	17.9	8	18.2
50–59	90	24.4	19	23.2	11	26.2	7	22.6	2	16.7	7	17.5	18	22.8	13	33.3	13	29.5
60–69	59	16.0	11	13.4	5	11.9	4	12.9	5	41.7	6	15.0	11	13.9	7	17.9	10	22.7
70–79	57	15.4	9	11.0	3	7.1	3	9.7	2	16.7	15	37.5	13	16.5	5	12.8	7	15.9
80–89	14	3.8	5	6.1	1	2.4			2	16.7	2	5.0	2	2.5	1	2.6	1	2.3
Not given	5	1.4	2	2.4			1	3.2			1	2.5			1	2.6		

note that aneurysms in the thorax were more frequently seen in laborers while those of the abdominal aorta or of the dissecting variety occurred more often in people having a sedentary occupation.

Etiology

Syphilis was the most common etiologic factor in the causation of aortic aneurysms in our series since 53.7 per cent were considered luetic. Arteriosclerosis was responsible for the formation of 21.4 per cent of the aneurysms and cystic medial necrosis accounted for 9.8 per cent. Table III presents an outline of the etiologic agents according to the morphologic type and the anatomical location. Several cases, however, deserve mention because of their uncommon etiology or unusual type. Three cases could be attributed definitely to a congenital defect. One was a 30-year-old white male with Marfan's syndrome who presented a well healed, dissecting aneurysm which involved the entire length of the aorta to produce a "double barrel" effect. Another was a 33-year-old white male with a large, congenital aneurysm of the sinus of Valsalva; and the third was an 8-year-old Negro male with a small (2 by 2 by 1 cm.) saccular aneurysm of the ascending arch which had ruptured into the pericardial cavity. An additional case of interest was that of a 40-year-old white male who died with advanced rheumatic fever. Near the right coronary ostia was a discrete aneurysm measuring 10 by 8 by 6 mm. and, although no inflammatory reaction existed within the aortic wall, the adjacent tissue was prominently involved by the rheumatic process. This man had a long rheumatic history and the cause of this aneurysm was believed to be either congenital or rheumatic, even though it is listed as "not determined" in Table III.

Morphologic Type and Size

Saccular aneurysm was the most common type in our series, occurring in 59.5 per cent of the 412 aneurysms. Table III lists the morphologic types of all the aneurysms according to the anatomical segment of the aorta in which they were found. The largest accurately recorded aneurysm in our series was a saccular lesion measuring 30 by 20 cm. located in the ascending arch; however, a majority of the aneurysms varied from 1 to 15 cm. in their greatest dimension. Nineteen per cent of the aneurysms measured less than 5 cm., 23 per cent varied from 5 to 10 cm., and 22 per cent were within the 10 to 15 cm. range. Single aneurysms involving more than one anatomical segment were larger in general than the average size, and the multiple aneurysms ordinarily were smaller than average size.

TABLE III

The Etiologic Factors and Morphologic Types of Aortic Aneurysms According to Anatomical Location (369 Cases)

Etiologic factors and morphologic types	Total frequency of etiologic factors (369 cases)		Frequency of etiologic factors and morphologic types in saccular and fusiform aneurysms												Dissecting aneurysms	
			Involving one anatomical segment only										Involving more than one anatomical segment		Frequency of etiologic factors and morphologic types, any location (44 cases)	
			Ascending aortic arch (82 cases)		Transverse aortic arch (42 cases)		Descending aortic arch (31 cases)		Descending thoracic (12 cases)		Abdominal aorta (40 cases)		Single lesion (79 cases)		Multiple lesions (30 cases)			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Syphilis	198	53.7	53	64.6	35	83.3	22	71.0	4	33.3	6	15.0	46	58.2	25	64.1	7	15.9
Arteriosclerosis	79	21.4	13	15.9	5	11.9	6	19.4	4	33.3	30	75.0	15	19.0	6	15.4		
Arteriosclerosis and lues	25	6.8	5	6.1					3	25.0			12	15.2	5	12.8		
Cystic medial necrosis	36	9.8															36	81.8
Congenital	3	0.8	2	2.4									1	1.3			1	2.3
Mycotic	4	1.1	2	2.4											1	2.6		
Not determined	24	6.5	7	8.5	2	4.8	3	9.6	1	8.3	4	10.0	5	6.3	2	5.1		
	Frequency of morphologic types (412 aneurysms)																	
Saccular	245	59.5	50	61.0	36	85.7	28	90.3	7	58.3	27	67.5	37	46.8	60	73.2		
Fusiform	114	27.7	30	36.6	6	14.3	3	9.7	4	33.3	10	25.0	40	50.6	21	25.6		
Dissecting	44	10.7															44	100
Not given	9	1.5	2	2.4					1	8.3	3	7.5	2	2.5	1	1.2		

Other Pathologic Findings

Erosion of bony structures (vertebrae, sternum, ribs, clavicle, costal cartilages) was encountered in 32 per cent of all cases studied and was categorically listed as absent in 51 per cent, with no mention of bony erosion being made in the remaining cases. Multiple aneurysms of the aorta and those involving the transverse and descending arch were more commonly responsible for erosion and it was found least frequently in the dissecting type.

A laminated thrombus within the aneurysmal sac was recorded as being present in 45 per cent of the cases and as absent in 24 per cent. No mention of a laminated thrombus was made in 31 per cent of the cases. A majority of the thrombi were found in aneurysms of the descending thoracic and abdominal segments and thrombi were frequent also in the multiple aneurysms of the aorta. Laminated thrombi were observed less frequently in aneurysms of the descending limb of the arch.

Incompetence of the aortic valves, insufficiency, and/or stenosis, was noted in 53 per cent of our cases and was seen most commonly in association with aneurysms of the ascending limb of the arch. The valves were normal in 29 per cent of the cases and no data were recorded concerning their status in 18 per cent.

Hypertrophy of the heart (over 375 gm.) was observed in 63 per cent of our cases, although the heart was actually atrophic in 5 per cent of the cases. A majority of hearts were hypertrophic regardless of the anatomical location or morphologic type of the aneurysm. This was notably true of aneurysms of the abdominal aorta and of dissecting aneurysms. Actually 80 per cent of the aneurysms of the abdominal aorta and 84 per cent of the dissecting aneurysms were associated with cardiac hypertrophy.

Clinical Features

The duration of illness is recorded in Table IV according to the anatomical location and morphologic type of the aneurysm. The average duration of illness in patients with syphilitic aneurysms was 24 months, with the shortest duration being less than 1 day and the longest 24 years. This latter case was that of a 64-year-old Negro male who had a calcified aneurysmal sac demonstrated roentgenologically in 1922. Subsequently he developed erosion of the 2nd to 6th thoracic vertebrae with transverse myelitis, and died of cardiac failure in 1946. The duration of symptoms in patients with arteriosclerotic aneurysms averaged 27 months, the shortest duration being less than 1 day

and the longest 11 years.

Table V summarizes the more prominent clinical findings according to the anatomical location and morphologic type of the aneurysm. The frequency of some signs and symptoms may be regarded as low, and perhaps we were too critical in our evaluation of clinical records; however, the figures listed are those in which the finding was unequivocal. In some cases clinical findings were listed as not given because of inherent difficulties in history taking, as previously mentioned, or because the patients were dead on arrival. No record of a previous hospital visit was found for many of these patients, but since this is the only charity service in the area and most of these patients were charity cases, it might be assumed that they had no serious complaint prior to their terminal episode. In a majority of instances the clinical manifestation of the aneurysm could be correlated directly with the anatomical location and morphologic type.

TABLE IV

The Duration of Illness in Patients with Aortic Aneurysms According to Morphologic Type and Anatomical Subdivision (369 Cases)

	Distribution according to duration of illness (369 cases)		Distribution as to duration of illness of saccular and fusiform aneurysms												Dissecting aneurysms Distribution as to duration of illness, any location (44 cases)	
			Involving one anatomical segment only										Involving more than one anatomical segment			
			Ascending aortic arch (82 cases)		Transverse aortic arch (42 cases)		Descending aortic arch (31 cases)		Descending thoracic (11 cases)		Abdominal aorta (40 cases)		Single lesion (70 cases)		Multiple lesions (39 cases)			
Time interval	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Less than 7 days	18	4.9	2	2.4					1	8.3	1	2.5	1	1.3			13	29.5
1 wk. to 1 mo.	32	8.7	9	11.0	6	14.3	5	16.1			2	5.0	3	3.8	2	5.1	5	11.4
1 mo. to 6 mos.	56	15.2	9	11.0	9	21.4	5	16.1	1	8.3	5	12.5	15	19.0	7	17.9	5	11.4
Over 6 mos.	112	30.3	24	29.3	9	21.4	6	19.3	5	41.7	14	35.0	29	36.7	17	43.6	8	18.2
Not given	151	40.9	38	46.3	18	42.9	15	48.4	5	41.7	18	45.0	31	39.2	13	33.3	13	29.5

Cause of Death

Rupture of the aneurysm was responsible for more deaths, 39 per cent, than any other cause and Table VI outlines the more common causes of death according to the anatomical location and morphologic type of the aneurysm. The various sites of rupture are listed in Table VII, where it can be noted that rupture into the pericardial cavity was the most frequent.

Comparison of the First and Last Groups of 100 Patients with Aortic Aneurysm

Additional data not readily demonstrable from an evaluation of the foregoing over-all case analysis were obtained when a comparison between the first 100 cases and the last 100 cases was made. As previously indicated, the first 100 consecutively recorded cases of aortic aneurysms were seen from 1892 to 1928 and the most recent 100 cases were observed from 1943 to 1953. In Table VIII significant variations are apparent with respect to incidence, location, etiologic factors, and age. Distribution as to sex and race was not appreciably different from that found in the entire group.

DISCUSSION

This study affords an analysis of aortic aneurysms encountered during a prolonged time interval and permits the comparison of 100 cases seen during a period of currently outmoded therapy with 100 cases observed within the era of antibiotics. Earlier diagnosis, better treatment, and a longer natural life span provide explanation for an older average age of the patients at necropsy and particularly those of syphilitic etiology (44.8 years in the first 100 cases and 61.0 years in the last 100 cases); a decrease in the over-all incidence of aneurysms from 4.36 per cent in the first 100 cases to 3.43 per cent in the last 100 cases; a decrease in the syphilitic type of aneurysm from 77 per cent in the first 100 cases to 49 per cent in the last 100 cases; a decrease in mycotic aneurysms so that none were seen from 1943 to 1953; and a prolongation of the duration of illness so that, in the more recent group, the average length of illness was 24 months in those cases of luetic origin and 27 months in the arteriosclerotic aneurysms. These life expectancy figures are similar to those of Lucké and Rea,[1] although Kampmeier[2] and Boyd[11] reported that the majority of patients died from 2 to 12 months following the onset of symptoms.

There has been an increase in arteriosclerotic aneurysms from 9 per cent in the first 100 cases to 27 per cent in the last 100 cases with a

TABLE V

The Clinical Manifestations of Aortic Aneurysms According to Morphologic Type and Anatomical Location (369 Cases)

Clinical findings	Total distribution as to clinical findings (369 cases)		Distribution of clinical findings in saccular and fusiform aneurysms												Dissecting aneurysms	
			Involving one anatomical segment only										Involving more than one anatomical segment				Distribution of clinical manifestations, any location (44 cases)	
			Ascending aortic arch (82 cases)		Transverse aortic arch (42 cases)		Descending aortic arch (31 cases)		Descending thoracic (12 cases)		Abdominal aorta (40 cases)		Single lesion (70 cases)		Multiple lesions (39 cases)			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Dyspnea	180	48.8	43	52.4	21	50.0	10	32.3	5	41.7	15	37.5	40	50.6	23	59.0	23	52.3
Pain	172	46.6	30	36.6	19	54.2	14	45.2	4	33.3	20	50.0	38	48.1	21	53.8	26	59.1
Cough	148	40.1	31	37.8	25	59.5	18	58.1	2	16.6	8	20.0	36	45.5	22	56.4	6	13.6
Dullness	80	21.7	17	20.7	14	33.3	8	25.8	3	25.0	7	17.5	14	17.7	12	30.8	5	11.4
Pulsating mass	68	18.4	9	11.0	15	35.7	5	16.1	1	8.3	10	25.0	16	20.2	8	20.5	4	9.1
Tracheal tug	49	13.3	10	12.2	12	28.6	5	16.1	1	8.3			11	13.9	7	17.9	3	6.8
Thrill	33	8.9	13	15.8	4	9.5	1	3.2	1	8.3	3	7.5	3	3.8	4	10.3	4	9.1
Aneurysmal murmur	30	8.1	7	8.5	2	4.8	1	3.2	1	8.3	3	7.5	8	10.1	5	12.8	3	6.8

consequent increase in aneurysms of the abdominal segment from 13 per cent to 22 per cent. Another notable increase has involved cystic medial necrosis, for in the first 100 cases only one instance was recorded as compared with 22 cases in the last 100 patients. This may account for the increased frequency of dissecting aneurysms, from 2 per cent in our first 100 cases to 22 per cent in the last 100 cases. In contrast to the over-all predominance of the Negro race in our series, dissecting aneurysms showed an even distribution between the white and colored race, while abdominal aneurysms were more commonly found in the white race, 67.5 per cent. Many patients with abdominal and dissecting aneurysms were noted to have a sedentary occupation.

Abdominal and dissecting aneurysms were associated with cardiac hypertrophy in a high percentage of our cases, 80 and 84 per cent, respectively. In some of these cases we were unable to establish satisfactorily the cause for cardiac hypertrophy on a valvular, renal, or arteriolosclerotic

TABLE VI

The Cause of Death in Patients with Aortic Aneurysms According to Morphologic Type and Anatomical Subdivision (369 Cases)

Cause of death	Distribution of total group as to cause of death (369 cases)		Distribution as to cause of death in saccular and fusiform aneurysms															Dissecting aneurysms Distribution as to cause of death, any location (44 cases)	
			Involving one anatomical segment only										Involving more than one anatomical segment					
			Ascending aortic arch (82 cases)		Transverse aortic arch (42 cases)		Descending aortic arch (31 cases)		Descending thoracic (12 cases)		Abdominal aorta (40 cases)		Single lesion (79 cases)		Multiple lesions (39 cases)			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Rupture	144	39.0	20	24.4	14	33.3	19	61.3	4	33.3	16	40.0	25	31.6	17	43.6	29	65.9
Pressure	15	4.1	2	2.4	4	9.5	1	3.2			2	5.0	4	5.1	2	5.1		
Heart failure	88	23.8	25	30.5	8	19.0	6	19.3	2	16.6	11	27.5	22	27.8	9	23.1	7	15.9
Pneumonia	37	10.0	9	11.0	4	9.5	1	3.2			2	5.0	10	12.7	6	15.4	3	6.8
Unrelated causes	85	23.0	26	31.7	12	28.6	4	12.9	6	50.0	9	22.5	18	22.8	5	12.8	5	11.4

TABLE VII

Rupture Sites of Aortic Aneurysms According to Morphologic Type and Anatomical Location
(144 Ruptures)

Sites of rupture	Total frequency of rupture (360 cases, 144 ruptures) No.	%	Frequency of rupture in saccular and fusiform aneurysms — Involving one anatomical segment only: Ascending aortic arch (82 cases, 20 ruptures) No.	Transverse aortic arch (42 cases, 14 ruptures) No.	Descending aortic arch (31 cases, 19 ruptures) No.	Descending thoracic (12 cases, 4 ruptures) No.	Abdominal aorta (40 cases, 16 ruptures) No.	Involving more than one anatomical segment: Single lesion (79 cases, 25 ruptures) No.	Multiple lesions (30 cases, 17 ruptures) No.	Dissecting aneurysms — Frequency of rupture, any anatomical segment (44 cases, 29 ruptures) No.
Pericardial cavity	41	28.5	9	2	1			7	3	19
Left pleural cavity	23	16.0	1	2	4		2	6	4	4
Left bronchus	17	11.8	1	3	8			3	2	
Peritoneal cavity	15	10.5					10	1	2	2
Right pleural cavity	12	8.3	2		1	2	1	2	2	2
Esophagus	9	6.2		3	3			2	1	
Mediastinum	5	3.5	1	1		2				1
Retroperitoneum	5	3.5					3		1	1
Superior vena cava	5	3.5	1	1				1	1	
Trachea	3	2.1		2				1		
Left lung	2	1.4			1				1	
Right lung	2	1.4	1		1					

* Each of the following sites was involved in a single rupture—from ascending arch: right auricle,[14] right bronchus, esophagus and right bronchus, esophagus and right bronchus; from an aneurysm involving more than one anatomical segment: right ventricle,[15] pulmonary artery;[16] and from multiple aneurysms: both lungs simultaneously.

basis. We did not encounter as many congenital lesions in our patients with dissecting aneurysms as might be expected from Gore's recent report,[12] and only 11.5 per cent of our dissecting aneurysms occurred before the age of 40, which is in contrast with the studies of Gore[12] and of Schnitker and Bayer.[13]

Multiple aneurysms of the aorta were seen in 10.6 per cent of our cases, and the data of others regarding the incidence of multiple aneurysms have shown an incidence which varied from less than 5 per cent[2] to 20 per cent.[1] The majority of our examples of multiple aneurysms consisted of two lesions only, but we had one aorta with four. Lucké and Rea[1] recorded one case with five aortic aneurysms. Although multiple aortic aneurysms did not appreciably alter the patient's prognosis, they occurred frequently enough to have clinical significance.

The clinical signs and symptoms presented by our cases are similar in frequency to those found in studies by Kampmeier,[2] Lemann,[4] and Boyd.[11] For some signs and symptoms the incidence in our series may

TABLE VIII

A Comparison of the First 100 Consecutively Recorded Cases of Aortic Aneurysm with the Last 100 Cases

	First 100 cases, 1892 to 1928	Last 100 cases, 1943 to 1953
Necropsy incidence	4.36%	3.43%
Location:		
Thoracic aorta	82%	68%
Abdominal aorta	13%	22%
Thoracic and abdominal	5%	10%
Etiologic basis		
Syphilis	77%	49%
Arteriosclerosis	9%	27%
Medial necrosis	1%	22%
Others	13%	2%
Age		
Over-all average	46.2 yrs.	61.8 yrs.
Average, syphilitic	44.8 yrs.	61.0 yrs.
Average, arteriosclerotic	71.0 yrs.	65.5 yrs.

be regarded as low, and perhaps we were too critical in the evaluation of the clinical records; the examples accepted were those in which the finding was unequivocal. It must be borne in mind that many of these lesions may be clinically silent and that an awareness of the diagnostic possibility of an aortic aneurysm will be necessary for an earlier recognition and subsequent better prognosis.

Rupture of the aneurysm was the most common cause of death in our cases, 39 per cent, and this figure is similar to that obtained by Lucké and Rea[1] although it is lower than the 52 per cent recorded by Boyd.[11] Patients with dissecting aneurysms died more frequently from rupture, 66 per cent, than those with any other type, even though our figure is slightly less than those found by Gore[8] and Peacock.[6] Pressure as a primary cause of death was found infrequently, 4.1 per cent, in our group; however, Kampmeier[2] and Hare and Holder[3] encountered it more often. We found cardiac failure, pneumonia, and death from entirely unrelated causes in general accord with other surveys.[2,11]

SUMMARY

A survey of 9,273 necropsies at the University of Texas Medical Branch revealed 412 aortic aneurysms to be present in 369 cases. The over-all incidence of necropsy cases having aortic aneurysm was 3.98 per cent, with the majority of the cases occurring in the fifth, sixth, and seventh decades; however, aneurysms of the arch tended to occur in younger patients whereas lesions of the abdominal aorta and multiple and dissecting aneurysms were found in older patients. Although aneurysms were more frequent in the Negro race, dissecting aneurysms demonstrated an equal racial distribution and abdominal aneurysms were more commonly found in the white race. Syphilis was the etiologic agent in over half the cases, 54 per cent. Aneurysms were most often saccular and usually involved only one anatomical segment of the aorta. Multiple and dissecting aneurysms were present in 11 and 12 per cent of the cases, respectively. An unexpected pathologic finding was cardiac hypertrophy in over 80 per cent of the cases with a dissecting or abdominal aneurysm. The average duration of illness was 2 years for patients with syphilitic aneurysms and 27 months for those who were arteriosclerotic. The occurrence of clinical signs and symptoms correlated well with the anatomical features of the aneurysms. While the immediate cause of death was varied, rupture of the aneurysm was the most common single cause, being found in 39 per cent of the cases.

A comparative study of the first 100 cases, observed from 1892 to 1928, and the last 100 cases, encountered during the period 1943 to 1953, indicated an increase in diagnostic acumen, better treatment, and a longer natural life span on the basis of the following trends: a decrease in the incidence of aortic aneurysms; an increase in aneurysms involving the abdominal aorta or both the abdominal and thoracic aorta; a decrease in aneurysms having syphilis as their etiology; an

increase of aortic aneurysms based upon arteriosclerosis or cystic medial necrosis; an increase in the average age of patients with all types of aortic aneurysms; an increase in the age of patients with syphilitic aneurysms; and a decrease in the average age of patients with arteriosclerotic aneurysms.

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EXTRAMEDULLARY PLASMACYTOMA *

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Plasmacytoma, or plasmoma, was mentioned by Unna in 1891 and first described by Schridde in 1905. It is a tumor composed almost exclusively of plasma cells arranged in clusters or sheets with a scant, delicate, supportive, connective tissue stroma. Plasma cell tumors are divided into four groups: (1) myelomatosis or multiple myeloma, (2) solitary myeloma of bone, (3) plasma cell leukemia, and (4) extramedullary plasmacytoma.

In spite of several recent reports, extramedullary plasmacytomas are comparatively rare. Several pathologic conditions have been described under the term plasmacytoma, and there has been some duplication in reporting individual cases. However, a reasonable search through the literature reveals a total of 161 authentic cases of extramedullary plasmacytoma (Table I). The strange nature and behavior of this tumor, its relationship to the other groups of plasma cell tumors, and the unusual clinical features and long follow-up of one case (19 years) have stimulated us to undertake this study.

Extramedullary plasma cell tumors occur in a wide variety of organs and tissues. The majority of these are in the walls of the upper air passages. However, there is hardly an organ in which a plasma cell tumor or plasmacytoma has not been described.

TABLE I
Reported Cases of Extramedullary Plasmacytoma, Excluding the Conjunctiva

Upper respiratory tract and oral cavity	126
Other organs	35
Total	161

Hellwig, in 1943, analyzed 127 cases of extramedullary plasmacytoma. Stout and Kenny, in 1949 and covering the period between 1905 and 1949, did an exhaustive study of 104 cases with tumors in the upper air passages and oral cavity, including 9 of their own. These publications, as well as numerous others, form the basis for the present study. We have added a few cases not included in Hellwig's or Stout and Kenney's series up to 1949 and all those reported during the period between 1949 and 1953.

Table II summarizes, according to location, 22 additional cases of extramedullary plasmacytoma of the upper air passages and oral

TABLE II

Summary of 22 Cases of Extramedullary Plasmacytoma of the Upper Air Passages and Oral Cavity, 1947 to 1956

Case	Site	Author and year	Age	Sex	Single	Multiple	Metastases		Duration before diagnosis	Course	Therapy	Remarks
							Lymph nodes	Other organs				
1	Nasopharynx	Waltner, 1947	38	M	+				25 yrs.	No recurrence in 2 yrs.		
2		Waltner, 1947	61	F	+			+	4 yrs.	No recurrence in 5 yrs., 8 mos.; died	Radiation	
3		Andersen, 1949	39	M	+				2 yrs.	Recurrence, died	Radiation	
4		Andersen, 1949	72	M	+		+		9 mos.	No recurrence	Surgery, radiation	
5		Andersen, 1949	77	F	+		+		1 yr.	Died 7 mos. later		
6		Rawson, 1950	53	M	+		+	+		Improvement for 9 mos.	Radiation, nitrogen mustard	
7		Fuerste, 1950	56	M		+		+		Died in 2 yrs.		
8		Dolin and Dewar, 1956	66	M	+				9 yrs.	Recurrence and symptom-free for 19 yrs.	Surgery, radiation	
9		Dolin and Dewar, 1956	65	M	+				3 mos.	Recurrence after 1 yr.	Radiation, surgery	

No.	Site	Author, year	Age	Sex					Duration	Course	Treatment	Remarks
10	Nasal cavity	Andersen, 1949	73	F	+					No recurrence in 4 yrs.	Radiation	
11		Rawson, 1950	83	F	+				4 wks.	No recurrence in 2 yrs.	Radiation	
12		Rawson, 1950	73	M	+	+				Recurrence after 5 yrs.; survival	Surgery, radiation	Recurred, treated by radiation
13		Rawson, 1950	72	M	+					No recurrence in 2 yrs.	Radiation	
14	Larynx	Bárány, (1937?)	73	M	+					1 yr. follow-up, no recurrence	Surgery	
15		Bárány, (1937?)	59	M	+					4 yrs., no recurrence		
16		Jaeger, 1942	74	M	+	+	+			3½ yrs.; died from intercurrent disease; no evidence of recurrence at necropsy	Radiation	
17		Rawson, 1950	59	M		+	+	+	2 wks.	10 yrs., 10 mos.; no recurrence		
18	Paranasal sinuses	Rawson, 1950	68	M	+						Radiation	No follow-up
19		Gupta, 1953	13	M	+				26 days		Radiation	No follow-up
20	Pharynx	Haines, 1942	75	M	+				9 mos.			No follow-up
21		Andersen, 1949	57	M	+				6 mos.	2 yrs., no recurrence	Surgery, radiation	
22	Palate	Gupta, 1953	80	F	+				8 mos.	2½ yrs.; died postoperatively from growth in maxilla	Surgery	

cavity in chronologic order as they appeared in the literature after 1947.

Table III summarizes, also in chronologic order, the cases of extramedullary plasmacytoma in organs other than the upper air passages and oral cavity published between 1909 and 1953.

Plasma cell tumors of the conjunctiva, another site of predilection for extramedullary plasmacytomas, are not included in this study. Forty-seven cases of plasmacytoma of the conjunctiva were cited by Hellwig. Details are scarce and the cases are difficult to evaluate. These tumors present a separate problem due to the controversy concerning their relationship to inflammatory processes, particularly trachoma, and the reader is referred to the ophthalmologic literature.

Report of Cases
Case 1

L. D. M. (U.H. no. 189–387), a white male, 66 years of age, was first seen at the University Hospitals on August 10, 1944. In 1935 he had noticed gradually increasing difficulty in breathing through his nose. He was told he had a "blood tumor," and "cancer paste" was applied to a mass in the nose. He was partially relieved of his symptoms. In July, 1941, he noticed swelling of the left ankle. The swelling increased gradually and a roentgenogram (Fig. 1) revealed an expanding, destructive lesion of the distal end of the tibia. On March 7, 1942, the tibial lesion was excised and the defect filled with bone grafts. Microscopic examination (Fig. 2) revealed a plasma cell tumor. Difficulty in breathing through the nose increased and on August 10, 1944, a brown-red tumor was noted, projecting from the nasal orifice, completely obstructing the nasal passages, and protruding posteriorly behind the uvula. The nasal bones and cartilage were destroyed, and the defect showed sharply demarcated edges, presumably the result of the "cancer paste." Biopsy of this tumor (Fig. 3) revealed a plasmacytoma.

The nasal tumor was treated by radiation: 2,400 r. in air to two fields in a period of 2 weeks. Ten weeks later, the nasal obstruction was relieved completely. However, there was still residual tumor present, and a second course of radiation therapy was given: 1,000 r. to two fields within a period of 1 week. When examined 6 months later, the oro-pharyngeal obstruction was relieved completely and no residual tumor was noted.

At that time the patient complained of pain and swelling in the left knee. A roentgenogram (Fig. 4) on September 11, 1945, revealed a destructive lesion invading the distal end of the femur. A mid-thigh amputation was performed. Microscopic examination again revealed a plasma cell tumor. The skeletal survey failed to reveal any other involvement.

Total serum proteins, albumin-globulin ratio, and all other laboratory tests were within normal limits. No Bence Jones protein was found. Five months later, the patient began to complain of pain and swelling in his right ankle. A roentgenogram revealed an expanding, rarefying lesion of the distal end of the right fibula. Repeated skeletal survey again failed to reveal other bone involvement. Laboratory tests, including those for Bence Jones proteinuria, were repeatedly negative. The lesion of the distal end of the right fibula was excised and the microscopic diagnosis was plasma cell tumor. The symptoms returned in the middle of 1951 and a recurrent plasma cell tumor of the distal end of the right fibula again was excised on February 27, 1952.

The patient was symptom-free until the end of 1952, when again he noted difficulty

TABLE III

Summary of 35 Cases of Extramedullary Plasmacytoma of Regions Other than the Upper Respiratory Area, 1909 to 1953

Case	Site	Author and year	Age	Sex	Single	Multiple	Metastases		Duration before diagnosis	Course	Therapy	Remarks
							Lymph nodes	Other organs				
1	Ileum	Vallone, 1930	24	M	+						Surgery	
2		North, 1930	47	F	+						Surgery	
3		Razzaboni, 1930				+					Surgery	
4		Brown and Liber, 1939	57	M		+	+	+	14 yrs.	Died post-operatively	Surgery	Also colon
5		Arel, 1946	8	M	+		+	+	6 mos.	Died 17 days postoperatively	Surgery	
6	Lymph nodes	Maresch, 1909	48	M	+			+	18 yrs.	Died post-operatively	Surgery	
7		Jackson, Parker, and Bethea, 1931	67	F	+					3 yr. follow-up	Surgery	
8		Jackson, Parker, and Bethea, 1931	53	M		+		+		Few weeks, died; no necropsy		
9		Basset and Scapier, 1937	48	M	+			+	2 yrs.	6 mos., died postoperatively	Radiation, surgery	

Table III (continued)

Case	Site	Author and year	Age	Sex	Single	Multiple	Metastases Lymph nodes	Metastases Other organs	Duration before diagnosis	Course	Therapy	Remarks
10	Stomach	Vasiliu and Popa, 1928				+	+					No details
11		Jaeger, 1942	45	F	+		+	+		4½ yrs., recurrence; 3½ yrs., no recurrence	Surgery, radiation	
12		Esposito and Stout, 1945	35	M		+	+		3 yrs.	2 yrs., died	Surgery, radiation	
13		Arel, 1946	15	M	+					Recurrence, died	Surgery, radiation	
14		Couret, 1946	48	F	+		+		8 yrs.	1 yr., died; recurrence	Surgery	
15		Schwander, Estes, and Cooper, 1947	42	M	+		+		6½ yrs.		Surgery	
16		Gupta, 1953	35	M	+					6 mos.	Surgery, radiation	
17	Lung	Gordon and Walker, 1944	30	F	+						Surgery, radiation	
18		Rozsa and Frieman, 1953	69	M	+					11 mos., no symptoms	Surgery, radiation	
19	Thyroid gland	Voegt, 1938			+						Surgery	
20		Shaw and Smith, 1940	50	F	+					1 yr. follow-up, no recurrence	Surgery, radiation	

No.	Site	Author, year	Age	Sex				Duration	Outcome	Treatment	Remarks
21	Submaxillary area	Aragona, 1936	6	F	+				5 yr. follow-up, no recurrence	Radiation, surgery	
22		Andersen, 1949	52	F	+				3½ yrs., no recurrence	Surgery	
23	Pleura	Klose, 1911	61	M	+				Died post-operatively	Surgery	
24	Mediastinum	Bros, 1931	54	M	+					Surgery	
25	Scalp	Wright, 1953	50	M	+					Surgery	
26	Skin	Hedinger, 1911	48	F	+	+		40 yrs.(?)		Surgery	
27	Spermatic cord	Ciaccio, 1913	38	M	+		+	2½ yrs.	8 yrs.	Surgery	
28	Vulva	Martinotti, 1910									Cited by Hellwig
29	Vagina	Appleberg, 1953	49	F	+	+			No recurrence	Surgery	
30	Breast	Cutler, 1934	66	F			+		20 mos.	Surgery, radiation	
31	Kidney	Knudsen, 1937			+				1 mo. follow-up, no recurrence		
32	Lacrimal gland	Parker, 1937	66	F	+				2 recurrences in 16 mo. follow-up	Surgery, radiation	Eroded maxillary bone
33	Ovary	Voegt, 1938	30	F	+					Surgery	
34	Cervix	Andersen, 1949	34	F	+			2 mos.	3½ yrs., no symptoms	Radiation	
35	Testicle	Andersen, 1949	72	M	+			2 mos.		Surgery, radiation	

in breathing through his nose. A recurrent red-brown nasopharyngeal tumor was found blocking the nasal passages. A biopsy of the tumor (Fig. 5) revealed plasmacytoma.

Another course of radiation therapy was administered: 2,000 r. in air to one field, 6 by 8 cm., within a period of 3 weeks. Skeletal survey again was negative. Total serum proteins, 8.3 gm. per 100 ml.; albumin-globulin ratio, 5.4/2.9. Electrophoretic determinations were normal. Serum calcium was 11 mg. per cent; serum phosphorus, 2.7 mg. per cent; serum alkaline phosphatase was within normal limits. No Bence Jones protein was found.

The patient was last seen on July 1, 1953. Examination revealed complete regression of the nasopharyngeal mass. Small, localized, indurated areas were noted in the nasal passages. Biopsy of an indurated area failed to show the presence of plasma cells. Results of laboratory tests were again within normal limits. A skeletal survey remained negative.

Case 2

A 65-year-old white male (U.H. no. 215–749) was admitted to the University Hospitals on July 9, 1953. He had noticed a nasal discharge 15 months before admission. Three months after admission, a tumor was found above the hard palate and biopsy showed it to be a plasmacytoma (Fig. 6). Radium needles were implanted, and this was repeated in October, 1953. The patient had no complaints until February, 1954, when he noticed an ulcer over his palate and burning pain ensued.

On admission, an ulcer through the hard palate, measuring 2 by 3 cm., was found. The defect exhibited folded, gray edges. Biopsy revealed recurrent plasmacytoma and osteonecrosis of the hard palate. Radical excision was performed. Laboratory tests including those for Bence Jones protein and serum proteins and a skeletal survey were negative. The patient is being followed currently.

DISTRIBUTION AND LOCATION

Haines, in a comprehensive study of malignant tumors of the upper air passages at Westminster Hospital, found that 50 to 90 per cent were epithelial tumors, the remainder being in the lymphosarcoma group. Plasma cell tumors were mentioned only occasionally by this author. Jaeger found 0.5 per cent of the malignant tumors of the upper air passages and oral cavity on file at the Roentgen Institute in Zurich to be plasmacytomas. It is evident that plasma cell tumors represent a rather small but nevertheless important fraction of the malignant tumors of the upper respiratory passages and oral cavity, and that hitherto they either have been confused with, or included in, the group of lymphosarcomas. Of all extramedullary plasmacytomas, 78.1 per cent occur in the upper air passages and oral cavity.

The distribution of plasmacytomas according to age and sex varies. Gormsen found 82 per cent in males between the ages of 40 and 70. Stout and Kenney found 80 per cent in males and the age distribution to be 10 to 89 years. An analysis of our tables reveals that 85 cases of extramedullary plasmacytoma of the upper air passages and oral cavity occurred in males, 22 in females, and in 19 cases the sex was not reported. This again reveals a 79.4 per cent preponderance for the male sex.

Of the 35 cases of extramedullary plasmacytoma in organs other than the upper air passages and oral cavity, 16 occurred in males and 14 in females. In 5 cases the sex was not reported. The age distribution ranged from 6 to 72 years.

Table IV presents the distribution of plasmacytomas of the upper air passages and oral cavity according to their location and site of metastases. This table indicates that 38 of the 126 cases metastasized to regional lymph nodes and/or to distant organs.

It is worthy of note that only 12 of the 113 cases, in respect to which this information was known, had multiple lesions (10.6 per cent). Only 2 of those had metastases. These figures vary slightly from those published by Andersen and approach closely those given by Stout and Kenney. It was found that metastases may occur to distant organs, bone, and skin without involving regional lymph nodes, and that regional lymph node involvement does not necessarily imply a bad

TABLE IV

Location and Distribution of Plasmacytomas of the Upper Air Passages and Oral Cavity

Site	Number	Single focus	Multiple foci	Single or multiple un-determined	Metastasis		
					Lymph nodes	Bones	Other organs
Nasal cavity	28	24	4		9	3	3
Nasopharynx	29	23	5	1	4	3	1
Paranasal sinuses	16	14		2	4	2	1
Tonsils	15	14		1	8	4	
Maxilla and gingiva	8	7	1				
Pharynx	7	6		1	3		
Palate	5	5			1		
Epiglottis and subglottis	5	4	1		2	1	2
Floor of mouth	2	2					
Posterior pillar	1		1				
Uvula	1	1					
Tongue	1	1					
"Nasal and paranasal sinuses" (Geschickter)	8			8			
Total	126	101	12	13			

prognosis. The tumor may be limited or localized to the regional lymph nodes in a large number of cases. Although the number of our cases is not great, the pattern of behavior is usually the same, and our findings concur with the views of many other investigators.

Table V presents the distribution of extramedullary plasmacytomas in organs other than the upper respiratory passages and oral cavity,

and shows their sites of metastases. In this group, 27 tumors were single, 6 were multiple, and 2 were undetermined in this respect. Eleven (31.4 per cent) of the 35 had metastasized to regional lymph nodes and/or distant organs. The majority of these, 7 cases (64 per cent), showed metastases to regional nodes only. Five (46 per cent) of the 11 cases with metastases had multiple lesions. Six (54 per cent) were single. Thus, 31.4 per cent of the plasmacytomas of other organs metastasized as compared to 30.1 per cent of the plasmacytomas of the upper air passages.

TABLE V

Distribution of Metastases in Extramedullary Plasmacytoma of Other Regions Than Upper Respiratory Tract and Oral Cavity

Site	Number of cases	Single focus	Multiple foci	Metas- tasis	Metastasis Lymph nodes	Bones	Other organs	Remarks
Ileum	5	3	2	1	1		1	
Lymph nodes	4	3	1	3	1		3	
Stomach	3	2	1	3	3			
Cecum	3	3		1	1		1	
Submaxillary area	2	2						
Lung	2	2						
Thyroid gland	2	2						
Jejunum	1		1	1	1			
Pleura	1	1						
Mediastinum	1	1						
Skull	1	1			1			
Skin	1	1		1				
Spermatic cord	1	1						No details
Vulva	1							
Vagina	1	1		1				
Breast	1		1		1			
Kidney	1	1						
Lacrimal gland	1	1						
Ovary	1	1						
Cervix	1	?	?					
Testicle	1	1						
Total	35	27	6	11*				

* Six single, 5 multiple.

SURVIVAL AND FOLLOW-UP

A careful analysis of the literature reveals scarcity of details and lack of accuracy in a great number of the reports. Of the 126 cases of plasmacytoma of the upper air passages and oral cavity, 44 were not

accurately described or followed. Hence, our analysis is limited to 82 cases.

Table VI shows the follow-up and survival of patients with plasmacytomas of the upper air passages and oral cavity. Of the 61 cases followed for not more than 4 years, the distribution as to status at time of follow-up and the percentage of the total 82 cases represented by each subgroup appears in Table VI. Of the 7 alive with evidences of the neoplasm, 5 had recurrence at the original site and 2 had metastases to bones. Of the 24 who had died from plasmacytoma in 4 years, 2 succumbed postoperatively and only one of the 24 showed evidence of metastases to distant organs. In the second line of Table VI a similar distribution is shown for the cases which were followed for the period of 5 to 10 years after onset.

In the literature, cases were reported which were followed for more than 10 years and a few of these require special mention. Rawson followed a case for 11 years and 10 months without evidence of recurrence or metastasis. Von Werdt, Wachter, Oppikofer, and Claiborn and Ferris followed cases for periods of 10½ to 14 years without any evidence of recurrence or metastasis. Piney and Riach followed a patient for 12 years, who then developed metastases to bones and died 1 year later. Jaeger followed a case for 25 years. This patient had four local

TABLE VI

Survival Rate of Patients with Plasmacytoma of the Upper Air Passages and Oral Cavity

	Number of cases	Alive, free of symptoms	Alive, with symptoms	Died from plasmacytoma	Died from intercurrent disease
1–4 years	61	22 cases (26.8%)*	7 cases (8.5%)	24 cases (29.3%)	8 cases (9.8%)
5–10 years or more	21	12 cases (14.6%)	4 cases (4.9%)	5 cases (6.1%)	
Not followed	44				
Total	126				

* All percentages refer to the 82 cases which were followed.

recurrences and multiple bone involvement, yet he was still alive and symptom-free. Figi, Broders, and Havens followed 2 cases for 12½ and 16 years, respectively, without evidence of disease. Stout and Kenney followed a case for 14½ years without evidence of recurrent disease. One of our cases had been followed for 19 years.

In Table VII are summarized the follow-up and survival data in 35 cases of extramedullary plasmacytoma of organs other than the upper air passages and oral cavity. Eight cases lacked details and follow-ups, so that only 27 could be evaluated properly. Therefore all percentages

are based on the 27 cases which were followed and the results appear in Table VII. Five of the 8 patients who died from plasmacytoma succumbed during or shortly after operation.

A comparison of Tables VI and VII indicates that the percentage of the patients alive and free of disease in the period of 1 to 4 years is greater in plasmacytomas of other organs than in those of the upper air passages and oral cavity, 48.1 per cent versus 26.8 per cent. However, this ratio is reversed, or actually close to unity (11.1 per cent

TABLE VII

Survival Rate of Patients with Plasmacytomas of Other Organs than Upper Air Passages and Oral Cavity

	Number of cases	Alive, free of symptoms	Alive, with symptoms	Died from plasmacytoma	Died from inter-current disease
1–4 years	21	13 cases (48.1%)*		8 cases (29.7%)	
5–10 years or more	6	3 cases (11.1%)	3 cases (11.1%)		
Not followed	8				
Total	35				

* All percentages are based on the 27 cases which were followed.

versus 14.6 per cent) as the longer follow-up period of 5 to 10 years is reached. Combination of Tables VI and VII indicates the survival rate of 109 cases of extramedullary plasmacytoma. Of 82 patients followed for 1 to 4 years, 35 were alive and free of disease (42.7 per cent). Of 27 patients followed for 5 to 10 or more years, 15 were alive and free of disease (55.6 per cent).

DISCUSSION AND CONCLUSIONS

Extramedullary plasmacytomas vary considerably in size, the diameter ranging from one to several centimeters. They usually are well limited, firm, and spherical, but they may be lobulated, pedunculated, or polypoid and show evidence of infiltration. The great majority are yellow-gray with a red cut surface, while some of the tumors have a blue-red appearance. Involved regional lymph nodes are firm, gray-white, and may measure up to 3 cm. The symptoms are those due to pressure and obstruction. The nasal cavity or nasopharynx may be obstructed completely; a feeling of fullness of the sinuses is a frequent symptom when the tumor involves them. In the gastrointestinal tract, obstruction is the predominant feature of the clinical picture. Bleeding is a frequent accompaniment and many of the tumors show ulceration. The clinical course is extremely variable. As to behavior, these tumors fall into several main categories: (1) Tumors which are solitary;

(2) Tumors which are infiltrative and destroy adjacent tissue; (3) Tumors which are prone to recur after inadequate removal or inadequate radiation therapy; (4) Tumors which, as a group, show regional lymph node metastases; (5) Tumors which metastasize to adjacent lymph nodes and other organs; (6) A solitary tumor in soft tissues, with one or more tumors in other organs, usually bones.

Microscopic Appearance

Marschalkó, in 1895, described the principal component of the plasmacytoma as a plasma cell. To the plasma cell he assigned four distinct characteristics, as follows: (1) The cells are round, oval, or polygonal with abundant basophilic cytoplasm. Inclusions are not distinct or constant and Russell bodies are not found. (2) The nucleus is distinctive, being eccentrically placed. (3) A paranuclear halo is present. (4) A small nucleus has five to eight deeply stained clumps of chromatin radially arranged at the nuclear membrane. It is this characteristic that suggested the term cartwheel nucleus. Actually, one does not always find these distinctive characteristics clearly drawn. The cells may show considerable atypism and variation in size. The radial clumps of chromatin in the nuclei are not always apparent. In many instances numerous mitotic figures are noted.

All investigators agree that there should be no difficulty in distinguishing true plasma cell neoplasms from plasma cell granulomas (Voegt; Jaeger; Stout and Kenney). A granuloma shows a variety of cells; there is a mixture of plasma cells, leukocytes, lymphocytes, and fibroblasts. Macrophages usually are present and there is a proliferation of blood vessels throughout the granulomatous structure. Many of the "plasmacytomas" of the conjunctiva would fall under this category, according to Chojnacki. A true plasma cell neoplasm, on the other hand, is composed of compact clusters or sheets of plasma cells with very little connective tissue stroma.

Hellwig believed that the more atypical the plasma cell, the greater the variation in its size, and the more mitotic figures present, the higher the degree of malignancy. Ringertz suggested that a more delicate reticular stroma would indicate a more rapidly growing and metastasizing tumor. He did not accept a close relationship between the relative maturity of the plasma cells and their behavior. It has been stressed by various authors that the microscopic appearance need not be an indication of behavior. According to Boyd, plasma cells of all types are but variations of one cell type, and the apparent variety is due merely to anaplastic changes in that one fundamental type. As to the origin of the plasma cell, Maximow, in 1928, considered that it is de-

rived from the lymphocyte and, accordingly, to him plasma cell tumors were simply a variety of lymphomas.

Klemperer and Rohr believed that the plasma cell is an abnormal hematic cell, the origin of which may be traced to the primitive reticulum cell of the bone marrow. Hayes concluded that neoplastic plasma cells are immature plasmacytes derived from reticulo-endothelial cells. Jaeger stated that extramedullary plasmacytomas arise from plasma cells in lymphatic tissue, while plasmacytomas of bone arise from plasma cells of the osseous medulla. Lymph node involvement in multiple myeloma is exceedingly rare, while lymph node involvement in extramedullary plasmacytoma is almost the rule. Most pathologists share the view that multiple myelomatosis is a generalized disease and that metastasis plays no part in its development.

Although there is far from universal agreement on the origin and nature of the plasma cell, most authors follow Maximow in accepting a close relationship between the plasma cell and the lymphocyte.

The behavior and the clinical picture of extramedullary plasmacytoma, in contrast to multiple myelomatosis or plasma cell leukemia, favor Jaeger's views in dividing those entities regardless of whether all plasma cells have the primitive lymphocyte as their mother cell. Autochthonous growth in extramedullary locations is the most probable explanation. Plasma cell leukemias appear to present an exaggeration of all the phenomena of multiple myelomatosis (Breitenbucher and Hertzog).

The plasma cells found in multiple myelomatosis may have the same appearance as those of extramedullary plasmacytoma, or may actually be the same cells. However, in multiple myelomatosis they are part of a generalized widespread systemic disease with a disturbance in protein metabolism. Magnus-Levy emphasized the close relationship between plasma cells and the formation of serum proteins. Smereker believed that in multiple myelomatosis the metabolic disturbance is influenced by a neurosecretory mechanism in the pathways between the hypothalamus and the neurohypophysis. Jeschal likewise considered that the changes in the form of crystal pods or basophilic staining of the plasma cells are associated with abnormal protein formation. Brasz pointed to the laying down of abnormal proteins—paraproteins —in the form of crystals in the reticulum cells of the bone marrow in multiple myelomatosis. Extramedullary plasmacytomas do not exhibit the manifestations of this disturbance in protein metabolism. From these facts the impression may be gained that extramedullary plasmacytoma can be considered as a separate, distinct entity. The term

extramedullary plasmacytoma should be applied only to cases in which disseminated myelomatosis can be ruled out by roentgenologic, hematologic, chemical, and morphologic investigations (Bichel and Kirketerp, and others).

A precocious lesion of multiple myelomatosis may appear to be solitary for some months. A number of authors have stressed the fact (Lumb, Willis) that plasma cell tumors commencing in a solitary form may subsequently become multiple myelomatosis. This is true for some cases of solitary myeloma of bone and for some cases of extramedullary plasmacytoma. The study of solitary myeloma of bone and its relationship to multiple myelomatosis is beyond the scope of this paper. However, as far as the extramedullary plasmacytomas are concerned, it is our opinion, after a careful study of 161 cases, that this possible relationship to systemic disease should not alter the idea of separating these entities.

Obviously, one may encounter great difficulty in classifying a case of extramedullary plasmacytoma exhibiting multiple metastases to bones. The clinical picture may resemble multiple myeloma. However, the plasma cells of the bone marrow do not take part in a widespread involvement and the signs of disturbance in protein metabolism are not manifest. Caution must likewise be taken in the evaluation of cases of multiple myelomatosis with occasional involvement of extramedullary soft tissues (Hayes).

Most authorities agree that the clinical picture rather than the microscopic appearance should form the final basis for the evaluation of extramedullary plasmacytoma as such. Once the histopathologic nature of the extramedullary tumor has been established, one should explore the possibility that it merely represents the first manifestation of multiple myelomatosis. Tests for Bence Jones proteinuria; determination of total serum proteins, and of the albumin-globulin ratio, including electrophoretic curves; bone marrow aspiration, and roentgenologic survey of the skeleton should constitute the essential parts of the clinical investigation. Only after all of these tests have been evaluated and have been found negative as to multiple myeloma, and the patient followed closely for a period of 1 to 2 years, may one classify a tumor as an extramedullary plasmacytoma. These tumors may fall into one of the previously mentioned six clinical groups. Extramedullary plasmacytomas sometimes are permanently cured by surgical excision or radiation therapy. On the other hand, the prognosis in multiple myelomatosis is extremely poor; the average survival is 2 to 3 years.

Our analysis of 161 cases of extramedullary plasmacytoma published in the literature from 1905 to 1953 reveals that of 109 which were followed, 50 patients were alive and symptom-free for a period of 1 to 10 years or more, thus indicating a survival rate of 45.9 per cent.

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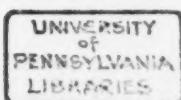
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LEGENDS FOR FIGURES

Fig. 1. Case 1. Roentgenogram of the left ankle. There is a large, destructive tumor at the distal end of the tibia with expansion and break-through of the cortex at the medial aspect and invasion of the soft tissues.

Fig. 2. Case 1. Photomicrograph of the tumor of the distal end of the tibia. Sheets and cords of cells with abundant cytoplasm and eccentric nuclei are shown. Most of the nuclei show "cartwheel" clumping of the chromatin. Some of the nuclei are dense.
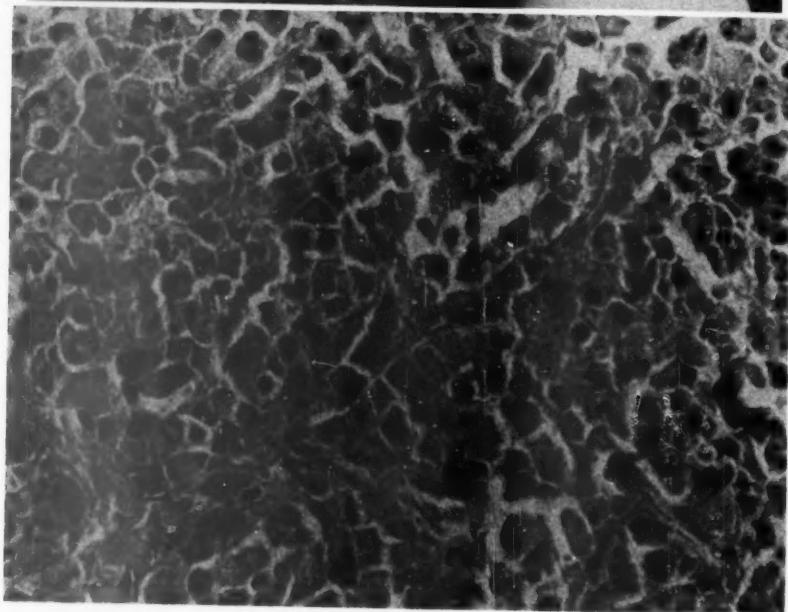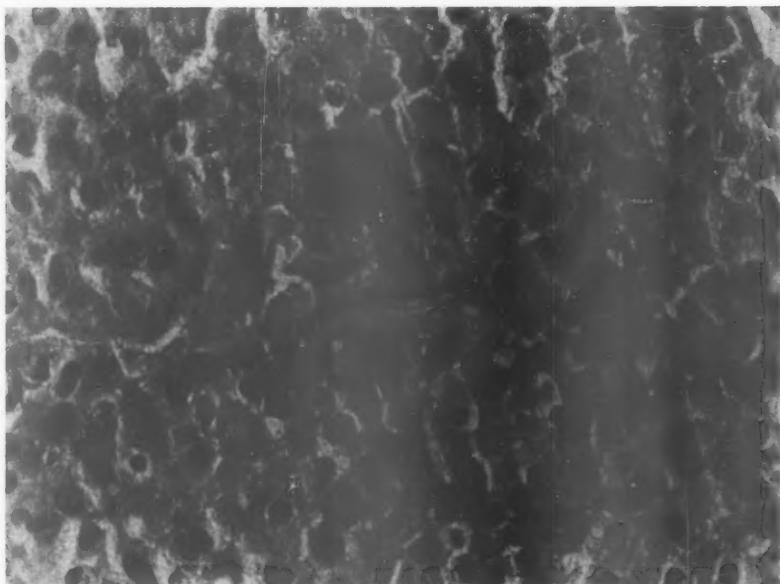
FIG. 3. Case 1. Photomicrograph of the nasopharyngeal tumor. Sheets and cords of cells with abundant cytoplasm and eccentric nuclei are demonstrated. Most of the nuclei are dense and hyperchromatic. Some of the nuclei show the "cart-wheel" clumping of the chromatin.

FIG. 4. Case 1. Roentgenogram of the left knee. An extensive destructive lesion is present at the distal end of the femur with a break in the cortex and invasion of the soft tissues at the lateral and posterior aspect.
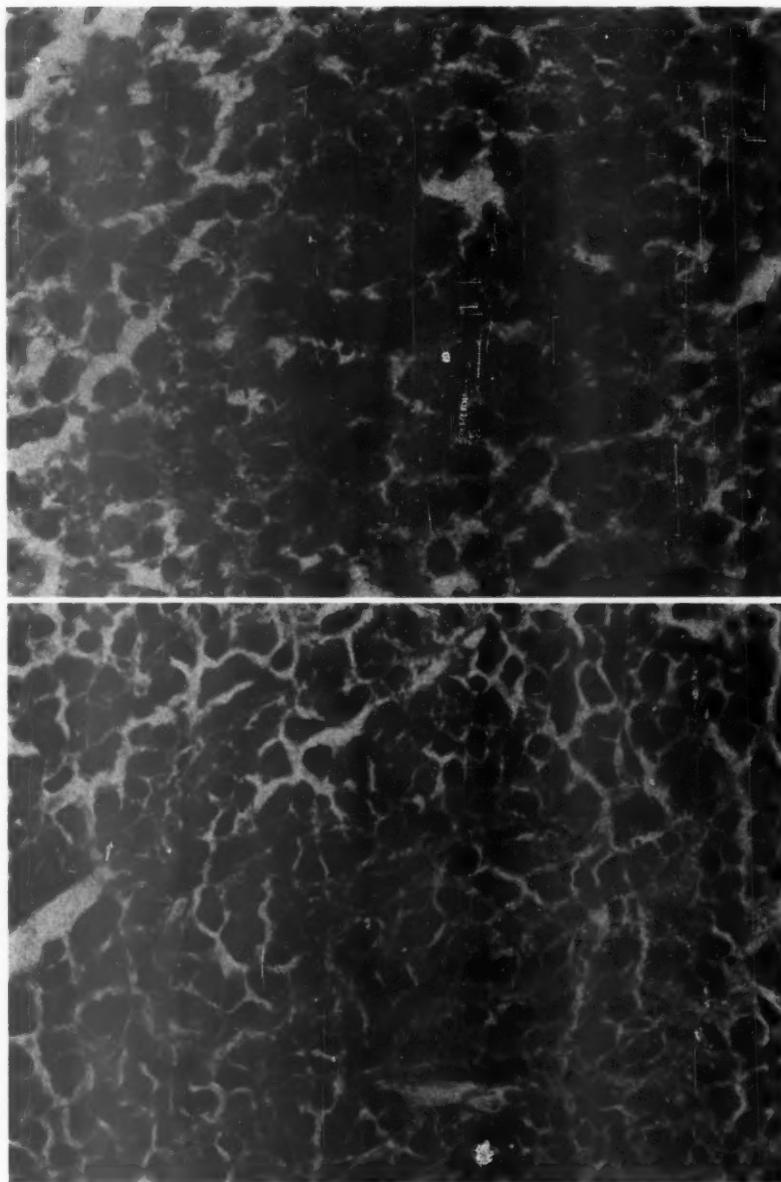
FIG. 5. Case 1. Photomicrograph of the nasopharyngeal recurrent tumor. The recurrent tumor shows a microscopic pattern similar to the original specimen (cf Fig. 3).

FIG. 6. Case 2. Photomicrograph of the nasopharyngeal tumor. Sheets and cords of cells with abundant cytoplasm and eccentric nuclei are present. Some of these are adjacent to small blood vessels. Most of the nuclei are dense and hyperchromatic.
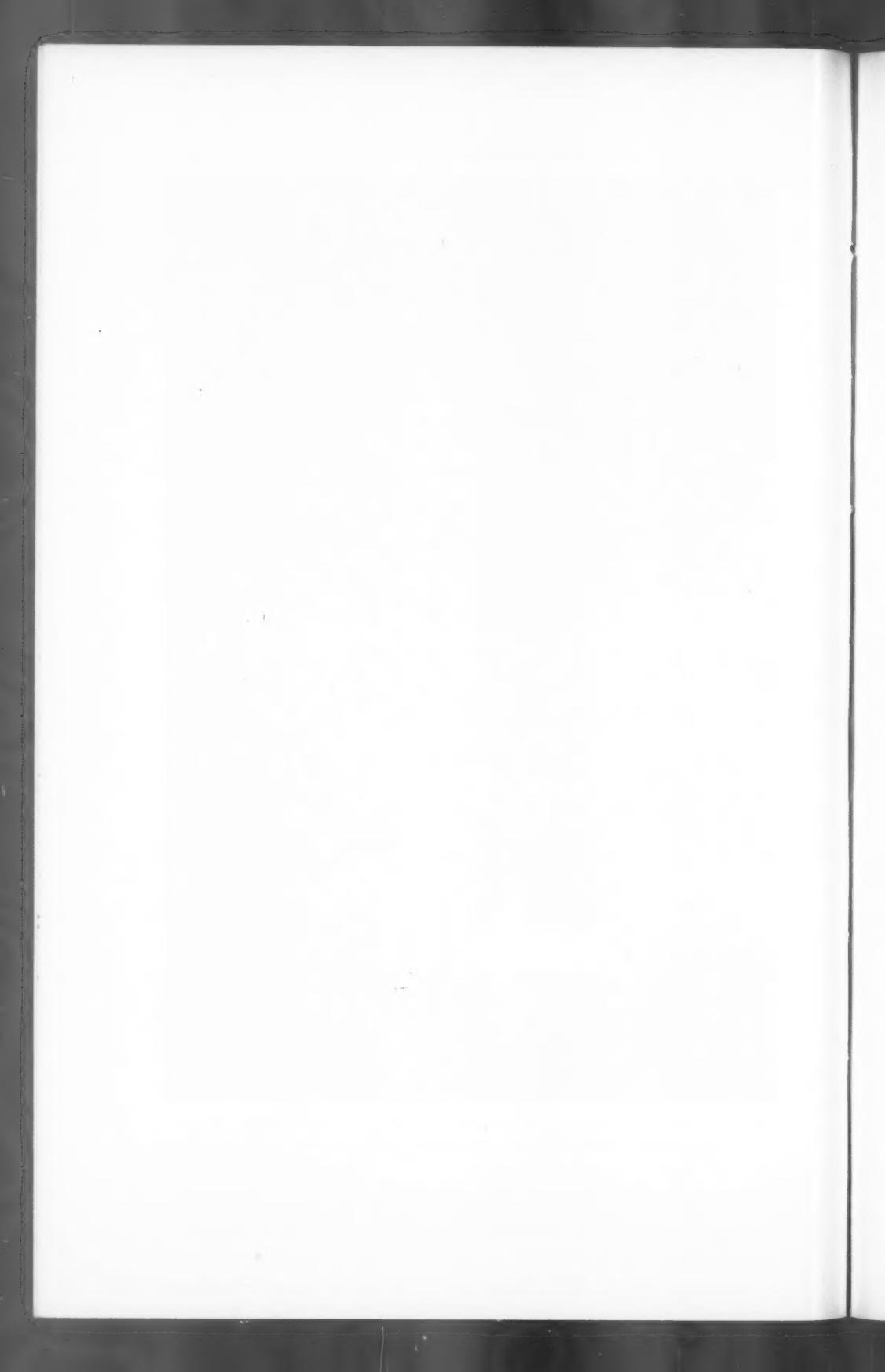
TESTICULAR DAMAGE FOLLOWING ETHIONINE ADMINISTRATION [*]

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The present report describes the damaging effects of ethionine, a methionine analogue, upon the testis of the albino rat. The sequence of changes in the testis during the administration and following the withdrawal of ethionine (alpha-amino-gamma-ethylmercaptobutyric acid) was studied and evaluated.

Material and Methods

Albino rats of the Sprague-Dawley strain were used. Each animal weighed approximately 220 gm. at the start of the experiment. The rats were fed ad libitum, a synthetic high-protein diet of the following percentage composition: glucose, 67; vitamin-free casein, 18; salt mixture, 4[1]; corn oil, 11 (containing 0.001 cc. haliver oil). Crystalline vitamins were added to supply the following amounts per 100 gm. of diet: thiamine chloride, 400 μg.; pyridoxine hydrochloride, 400 μg.; riboflavin, 800 μg.; calcium pantothenate, 1.5 mg.; and nicotinic acid, 2.5 mg. Five-tenths per cent of DL-ethionine was added to the diet of the experimental groups. Since impairment of weight gain was anticipated in the animals receiving ethionine, an additional control group was fed the basic diet, in limited amounts, in order to match the weight change in the ethionine-fed groups. The animals were weighed twice a week and were killed after 10, 28, and 63 days. The ethionine was removed from the diet of one group of 8 animals after 28 days and the degree of regeneration was studied 35 days later.

The tissues were fixed in formalin, sectioned, and then stained with hematoxylin and eosin. The changes in the testes were graded histologically from 0 to 5. In grade 0 no changes were seen (Fig. 1). Testes with a decrease in the number of spermatozoa and some degeneration of the spermatids were graded as 1 (Fig. 2). In grade 2, there was a marked decrease in the number of spermatozoa with marked degeneration of the spermatids, only a few tubules remaining intact (Fig. 3). In those graded 3, all tubules were affected. There was almost complete absence of spermatozoa, a decrease in the number of spermatocytes, and occasional degeneration of spermatogonia (Fig. 4). In the testes graded 4, only spermatogonia were seen in most tubules, although in

some, degenerated spermatids and spermatocytes were still present (Fig. 5); while in grade 5 only Sertoli cells were present in most tubules, with occasional spermatogonia in some (Fig. 6).

RESULTS

After 10 days (Table I), each animal in the ethionine supplemented group showed grade 1 degeneration of the testes, whereas the control

TABLE I
Extent of Testicular Damage with Ethionine

Day of experiment	Dietary group	Number of animals	Average grade of damage	Average weight change
				%
	Basic+0.5% ethionine	4	1	−19
10	Basic (limited intake)	4	0	−21
	Basic	4	0	+27
	Basic+0.5% ethionine	15	3.3	+6
28	Basic (limited intake)	7	0	+4
	Basic	15	0	+38
	Basic+0.5% ethionine	22	4.9	+1
63	Basic (limited intake)	8	0.37	−14
	Basic	13	0.3	+58

animals on the basic diet not supplemented by ethionine showed no testicular damage. The weight loss in the experimental group was 19 per cent, while in the control rats on a limited diet it was 21 per cent. After 28 days, testicular damage was seen in all 15 animals in the experimental group: 3 animals had grade 2 degeneration; 5, grade 3; 7, grade 4. For the entire group the average grade was 3.3. There was no testicular degeneration in the control group nor in the control group on a limited dietary intake. The average weight gain in the latter was 4 per cent and in the experimental group, 6 per cent. After 63 days there was marked testicular degeneration in all 22 animals in the group receiving ethionine: 3 were graded 4 and 19 were graded 5, with an average grade of 4.9 for the entire group. Only 3 of the 8 animals in the control group on limited dietary intake showed slight degeneration, with an average grade of degeneration of 0.37 for the entire group. In the control group on the ad libitum feeding, 2 of 13 animals showed grade 2 degeneration, with an average of 0.3 for the entire group. The average gain in weight for the experimental group was 1 per cent, while the average loss in the control group on a limited dietary intake was 14 per cent.

In the testes graded 1 (Fig. 2), in addition to the decrease in the number of spermatozoa and some degeneration of the spermatids, there was also interstitial edema. In situations where edema was not apparent, the tubules appeared to be closely crowded. As the lesion progressed in severity to grade 2 (Fig. 3), there was a marked decrease in the number of spermatozoa and a marked degeneration of the spermatids. Only a few tubules remained intact. In some tubules there was also degeneration of the primary and secondary spermatocytes, although this was not a striking feature at this stage. In grade 3 degeneration all tubules were affected to a greater or lesser extent (Fig. 4). In addition to these changes, there was a decrease in the number of spermatocytes and occasional degeneration of spermatogonia. Karyorrhexis, karyolysis, and pyknosis of the spermatocytes and some of the spermatogonia were noted. Occasionally a few giant cells were seen within the tubules. In the more advanced lesions (grade 4), although a few spermatids and spermatocytes sometimes were seen, the predominant germinal cells were the spermatogonia. Multinuclear giant cells were present in all testes in this group and this was one of the striking features (Fig. 5). In the most advanced lesions (grade 5), only Sertoli cells were present in most tubules (Fig. 6), with occasional spermatogonia in some. It is of interest that at this stage, with almost entire loss of germinal cells, there was a concomitant absence of the giant cells described in group 4, although a few were still present. There was no histologic evidence of damage to the Sertoli cells.

As the damage progressed there was alteration in shape and size of the seminiferous tubules. In the early stages (grade 1) the tubules became polyhedral or angulated rather than circular in cross section. In only one animal graded 0 were tubular angulation and slight edema noted, but with no cellular changes. However, as in the control, only one entire cross section of a tubule could be seen per high-power field (using a 10× wide-field eyepiece and a 43× 4 mm. objective, NA of 0.65). Later (grade 2), this arrangement disappeared and the tubules again became circular. However, they were decreased in size as seen on cross section, to the extent that in grade 3 two entire cross sections of a tubule could be seen in a high-power field. In grade 4, three tubules per high-power field were noted, while in grade 5, four, five, and even six tubules were present. In the latter two groups (grades 4 and 5), with the marked decrease in size of the tubules there was a wrinkling of the tunica propria. The interstitial cells of Leydig apparently were not altered histologically, but were more prominent and obvious in the atrophic testes.

Although there was no alteration in the epithelial lining of tubules

of the epididymis, the contents reflected the changes in the seminiferous tubules. In grade 1, the epididymis resembled its normal state very closely in that it contained many mature spermatozoa. However, a few spermatids were noted. In grade 2, although the cell population was still predominantly spermatozoa, the spermatids were more prominent. In grade 3 only occasional spermatozoa were present while the spermatids and spermatocytes were more prominent, and the total cell population was markedly decreased. In grades 4 and 5 the proximal portion of the epididymis was empty, whereas cells were still present in the distal portion. Most of the cells were immature and degenerated and the giant cells present contained degenerated nuclei. However, in the vas deferens some normal, mature spermatozoa were still observed.

In the control animals and in those with lesser degrees of tubular damage, the seminal vesicles were covered by a tall columnar epithelium with abundant cytoplasm. In animals in which testicular damage was marked, grades 4 and 5, the vesicular epithelium became atrophic and cuboidal with an over-all decrease in cytoplasm.

Associated changes in the prostate gland were not striking. In some cases, the glands were empty of secretion, somewhat shriveled and thin, and the epithelial lining was lower, varying from cuboidal to flat. However, there was no correlation between the degree of testicular degeneration and prostatic alteration.

All of the animals that were on the 0.5 per cent ethionine supplement for the first 28 days of the experiment and then placed on the control diet for 35 days showed testicular regeneration when compared with corresponding animals on ethionine for 28 and 63 days. The amount of regeneration in each testis varied from tubule to tubule, most of the tubules reaching the stage of spermatid formation, while in a few, spermatozoa were present (Fig. 7). A small number of tubules were found in which little or no regeneration had occurred. However, in the latter, no degenerating cells or débris were noted except for giant cells in one of the regenerating testes. The prostatic epithelium and the epithelium of the seminiferous tubules had returned to their original normal appearance.

DISCUSSION

Testicular changes have been described when other essential amino acids (phenylalanine,[2,3] threonine,[4] histidine,[5] leucine,[6] tryptophane[7]) had been withheld from the diet. It may be that methionine also is essential in spermatogenesis since marked testicular damage and loss of spermatogenesis were noted in animals receiving a diet supplemented with antimetabolite ethionine. The ethionine may have a direct effect on the condition of the seminiferous tubules, since some of the ethio-

nine labeled with radioactive sulfur (S[35]) becomes localized in the testis when fed to animals.[8] Although all of the animals fed ethionine developed pancreatic damage similar to that previously reported,[9-15] there was no correlation between the amount of pancreatic damage and the amount of testicular degeneration. This may indicate that the testicular change was not a result of the pancreatic damage, but rather independent of it.

An additional control group on a limited diet was used so that the weight change would match or exceed the weight change in the experimental animals. At the end of 28 days there was marked testicular degeneration in the experimental animals, whereas there was no damage whatsoever in the group on the limited dietary intake. The latter group gained an average of 4 per cent over the original weight, while the ethionine-fed group gained 6 per cent (Table I). After 63 days the control group and the group on the limited dietary intake showed some testicular damage in a few of the animals, but the changes by no means reached the marked damage present in the ethionine group. In the latter there was an average weight gain of 1 per cent, while in the group on the limited diet the weight loss was 14 per cent. This indicates that the testicular damage was due to the ethionine and was not the result of inanition.

When testicular damage was extensive (grade 4), multinucleated giant cells were a constant feature and were present in large numbers in the seminiferous tubules, although Alvizouri and Warren[9] reported that these cells occurred only rarely after ethionine administration. However, when tubular damage progressed to the extent that a few or no germinal cells were present, the giant cells also disappeared (grade 5). In some of the giant cells the nuclei resembled those found in the spermatids, while in others the nuclei were similar to those seen in spermatocytes. According to Siperstein,[16] Bouin and Garnier[17] considered that the giant cells resulted from abnormal mitosis of the nuclei within a cell; however, Siperstein believed that they are produced by fusion of degenerating primary spermatocytes, while Alvizouri and Warren claimed that they are formed by agglutination of degenerated spermatids. In our experiment the giant cells and the spermatogonia were the last to disappear. This would suggest that the giant cells are formed not by fusion of highly developed cells such as the spermatids, but by nuclear division and maturation to the point that some of the nuclei resembled those found in the spermatids, while others resembled those in the spermatocytes. At the same time, cytoplasmic division does not occur and the cells remain multinucleated.

The atrophy of the epithelial cells lining the seminal vesicles and the

prostate is of interest. The question arises whether these changes were secondary to the testicular damage or were due to the direct effects of ethionine. No obvious histologic changes were seen in the interstitial cells of Leydig. However, it is possible that the function of the Leydig cells may have been affected. When ethionine was removed from the diet and the testes had regenerated, there was also a return to normal of the epithelium of the prostate and seminal vesicles.

SUMMARY

Testicular damage was produced in albino rats when the diet was supplemented by 0.5 per cent DL-ethionine, indicating that methionine is essential in spermatogenesis. When the ethionine was removed from the diet, testicular regeneration was observed.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

Figs. 1 to 6. Sections of testes, illustrating varying degrees of degeneration of the seminiferous tubules. Hematoxylin and eosin stain. × 360.

FIG. 1. From control animal, graded 0. Apparently normal histologically.

FIG. 2. Ten days of ethionine feeding, grade 1 degeneration. Loss of spermatozoa, crowding of tubules, and interstitial edema.

FIG. 3. Twenty-eight days of ethionine feeding, grade 2 degeneration. Absence of spermatozoa, degeneration and decrease in number of spermatids.
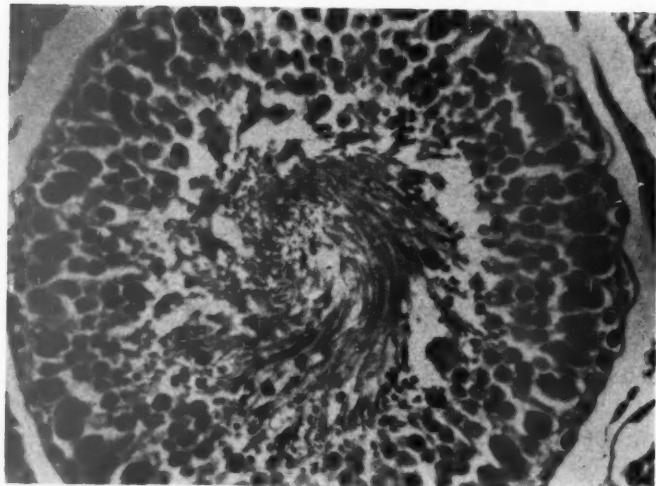
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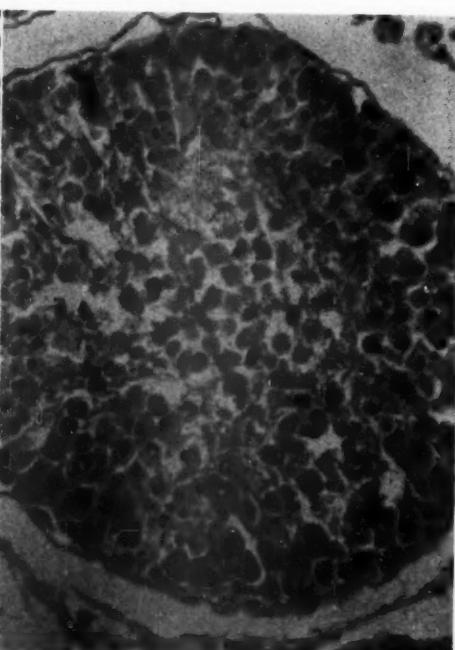
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FIG. 4. Twenty-eight days of ethionine feeding, grade 3 degeneration. Absence of spermatozoa and almost complete absence of spermatids. There are also a decreased number of spermatocytes and degeneration of a few spermatogonia.

FIG. 5. Twenty-eight days of ethionine feeding, grade 4 degeneration. Multinucleated giant cells are prominent. Absence of the germinal cells except for the peripheral spermatogonia. The diameter of the tubule is decreased and the tunica propria is wrinkled.

FIG. 6. Sixty-three days of ethionine feeding, grade 5 degeneration. The multinucleated giant cells are now absent. Sertoli cells at the periphery are the only cells within the tubule.

FIG. 7. Regenerating testis, 63 days after experiment started and 35 days after ethionine feeding ceased. The tubules shown are in different stages of spermatogenesis. Hematoxylin and eosin stain. × 150.
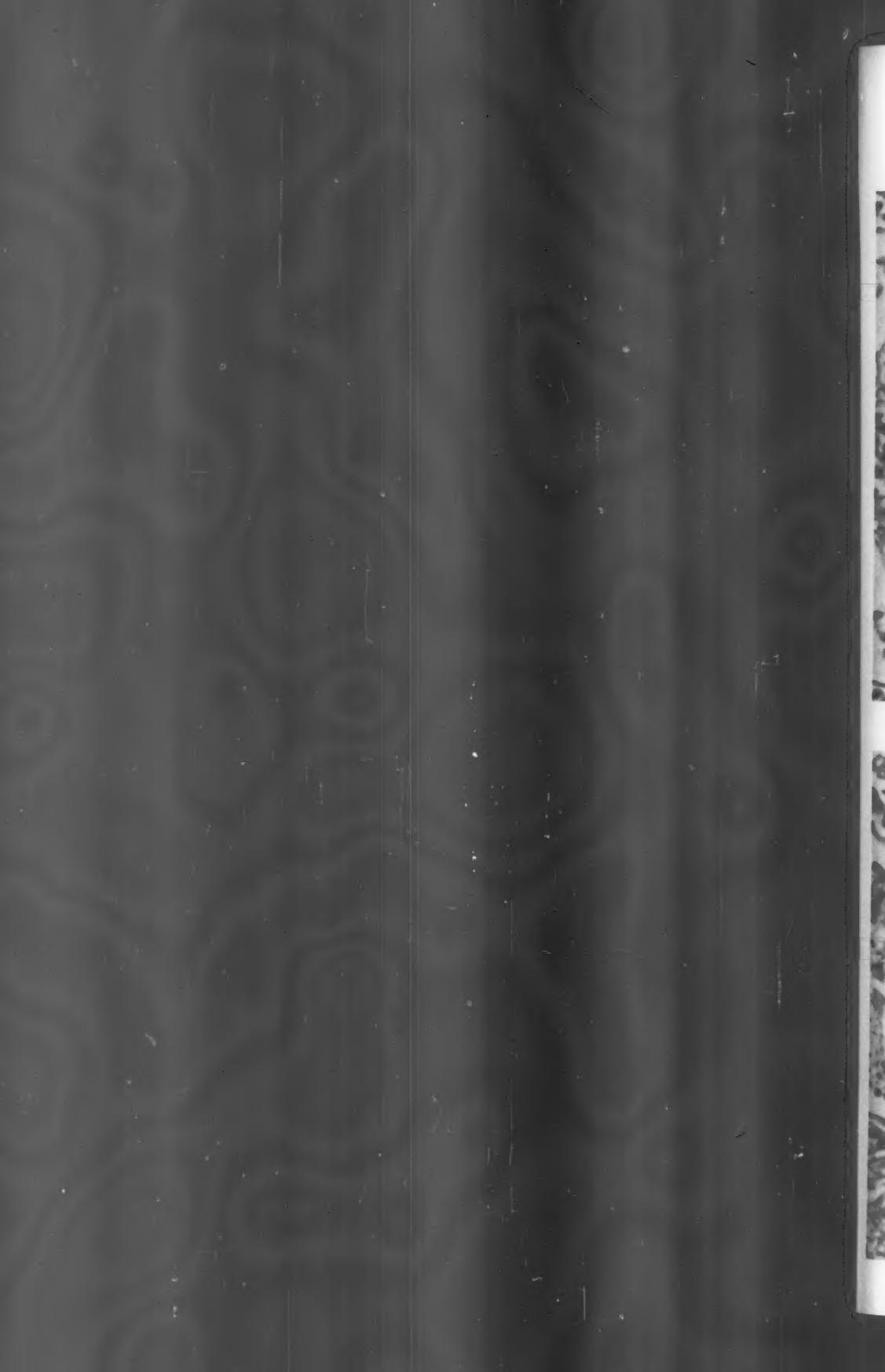
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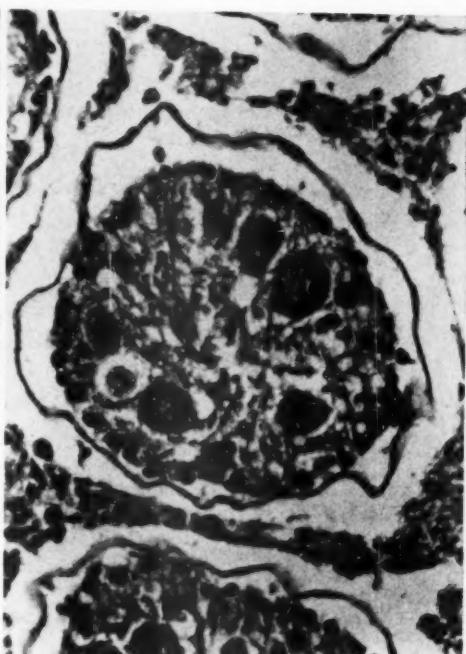
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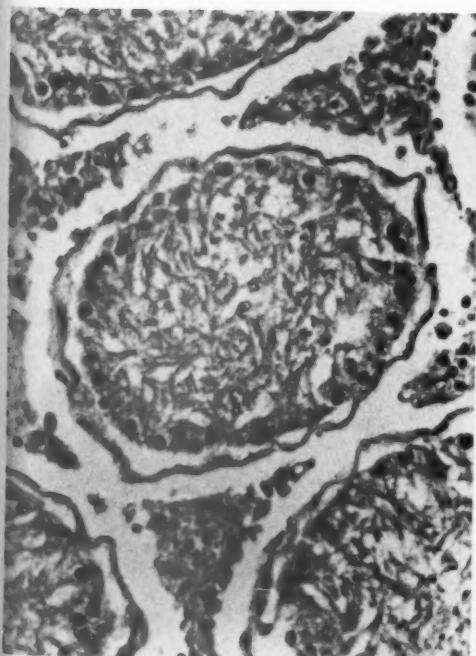
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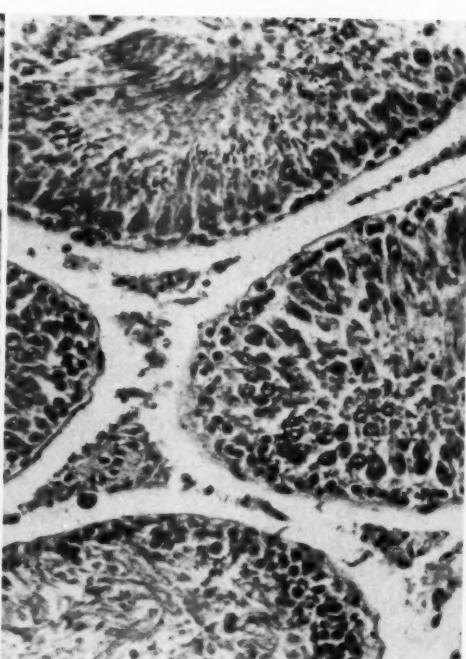
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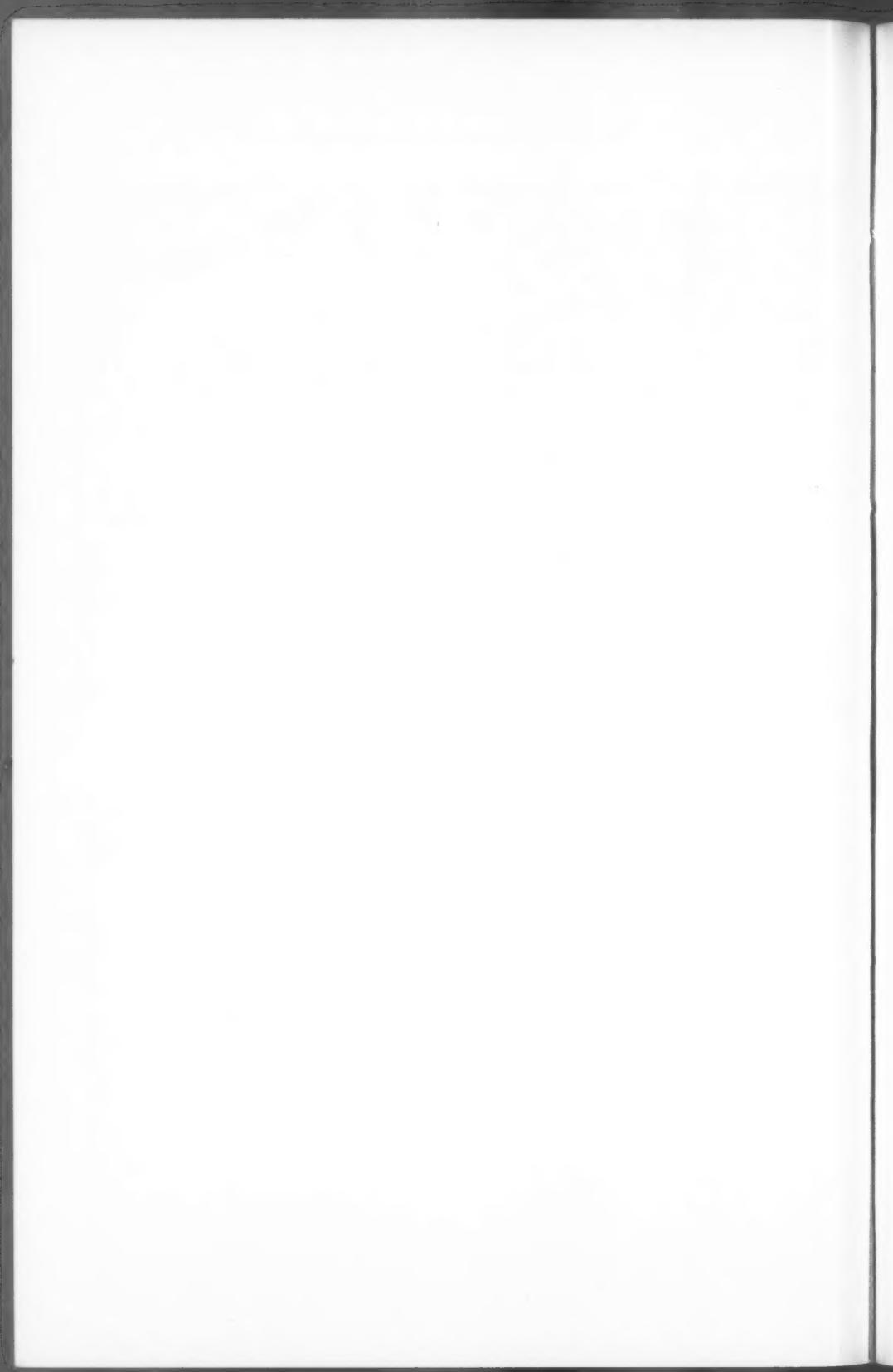
LESIONS INDUCED IN C57BR MICE WITH GALLIUM CITRATE AND METHYLCHOLANTHRENE *

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Radiogallium has been suggested as a therapeutic agent for osteogenic sarcoma by Dudley and Maddox,[1] Dudley *et al.*,[2] and Lang,[3] and as a means for diagnosing bone tumors by Mulry and Dudley.[4] Therapeutic trials of radiogallium have been reported by King *et al.*[5] and a comprehensive study of radiogallium and chemical gallium has been presented by Brucer and his associates.[6] Some studies on the acute and chronic toxicity of gallium citrate[7,8] have been reported. It is recognized that the tolerated dose of gallium citrate and the degree of its toxicity vary from species to species. However, it is of interest to study histologic changes brought about by long-term administration of tolerated doses of gallium citrate.

Eyestone[9] observed extensive changes in the gastric glandular mucosa of mice receiving one massive dose of gallium citrate. This result suggested the present experiment, for it was hoped that if 20-methylcholanthrene was added after the gastric lesion had been induced by gallium, gastric adenocarcinoma might be induced. This paper describes the lesions developing after repeated simultaneous administration of small doses of gallium citrate and methylcholanthrene to mice.

MATERIALS AND METHODS

C57Br mice between 2 and 6 months of age were divided into six groups. Each mouse received a subcutaneous injection of gallium citrate in aqueous solution (pH 6.5 to 7.0) once every week. All groups but one (group F) received 20-methylcholanthrene by mouth. The number and sex of animals in each group and the treatment they received are presented in Table I. Animals in groups A and B received 40 mg. per kg. of gallium; groups C and D, 60 and 80 mg. per kg., respectively; and groups E and F, 100 mg. per kg., as gallium citrate solution in a single injection per week. The concentration of the solution was so arranged that each animal received between 0.15 and 0.4 ml. of the solution per injection, depending upon the weight of the animal and dosage.

Mice in group A received 10 mg. per cent of methylcholanthrene in their diet. A benzene solution of the hydrocarbon was mixed with the semisynthetic diet[10] and the solvent removed from the diet by evaporation in warm air. Mice in groups B, C, D, and E were tube fed with a 30 mg. per cent solution of methylcholanthrene in polyethylene glycol,

TABLE I
Number of Mice in Each Group and Their Treatment

Group	No. of mice	Sex	Gallium		Methylcholanthrene		Duration of treatment Ga/MCA
			mg./kg./wk.	Total mg.	mg./wk.	Total mg.	wks.
A	15	F	40	17.6	1.4*	30.8	22/22
B	15	F	40	17.6	0.27	5.9	22/22
C	14	M	60	33.6	0.27	9.2	28/34
D	14	F	80	44.8	0.27	9.2	28/34
E	7	F	100	56.0	0.27	9.2	28/34
F	6	M	100	56.0			28/

Gallium citrate solution was injected subcutaneously once a week.

    * In this group 20-methylcholanthrene was dissolved in benzene and then mixed in the synthetic diet, at the rate of 10 mg. per 100 gm. of diet. The amount of methylcholanthrene consumed per week and the total are calculated on the assumption that a mouse consumes about 2 gm. of food per day. In all other groups, animals received methylcholanthrene by stomach tube; 0.3 ml. of 30 mg. per cent solution of methylcholanthrene in polyethylene glycol, 3 times a week.

three times a week, with 0.3 ml. of the solution per feeding. Animals in group F received no methylcholanthrene. All mice except those in group A were fed on Purina laboratory chow pellets.

A limited study of the hematopoietic response was made. Red, white, and differential counts were made on 3 animals from each group immediately before, and 24 hours after, a gallium injection, and at the time of necropsy.

Animals were killed at intervals and sections were made of the lungs, liver, spleen, stomach, pancreas, adrenal glands, and gonads, and any other organs that showed gross changes. The tissues were fixed in 10 per cent formalin, Zenker's acetic, or Zenker's formol solutions. Microscopic sections were stained routinely with hematoxylin and eosin.

RESULTS

All mice showed alopecia at the site of the gallium citrate injections. The lesions produced in mice receiving various doses of gallium and methylcholanthrene are presented in Table II. All doses of gallium induced the same kind of lesions and there was no significant difference in the lesions produced by methylcholanthrene ingested in food

and by that given by stomach tube. Animals in groups receiving a small dose of gallium had a longer life span than animals in groups receiving a high dose of gallium. Thus, the average life span for group A, which received 40 mg. per kg. of gallium and methylcholanthrene, was 12 months, while that for group E, which received 100 mg. per

TABLE II

Tabulation of the Lesions Found According to Groups

Group	No. of mice	Forestomach lesions			Lesions of glandular stomach	Renal lesions	Duodenal lesions
		Hyp.	Pap.	S.c.c.			
A	15	1	2	4	8	12	4
B	15	6	2	1	10	10	1
C	14	2	2	9	9	4	
D	14	3	3	7	10	4	
E	7	1	2	1	4		
F	6				1	1	
Total	71	13	11	22	42	31	5

Hyp. = hyperkeratosis; Pap. = papilloma; S.c.c. = squamous cell carcinoma.
Lesions of the glandular stomach were destruction of parietal and chief cells.
Lesions of the kidney were cortical atrophy and tubular degeneration.
Lesions of the duodenum were atrophy, hyperplasia, and polyps.

kg. of gallium and methylcholanthrene, was 3.7 months. The mice of group F, which received 100 mg. per kg. of gallium only, lived for about 8 months.

The glandular stomach in mice in every group showed lesions which consisted of destruction followed by regeneration of the parietal and chief cells. Atrophy of the cortex of the kidney with degeneration of the epithelium of the tubules also was noted in mice from most of the groups. Lesions of the forestomach, including hyperkeratosis, papillomas, and squamous cell carcinomas, were found in every group receiving methylcholanthrene. Five mice receiving methylcholanthrene and 40 mg. per kg. of gallium, which lived for a year or more after the start of the experiment and were 16 months or older at the time of necropsy, showed atrophy of areas of the duodenal mucosa and extensive adenomatous growth in other areas in the duodenum.

Hematopoietic System *

A study of the peripheral blood, lymph nodes, and spleen did not show significant alterations which could be attributed to either gallium or methylcholanthrene. Significant alterations from the normal were

* We are grateful to Dr. Thelma B. Dunn for these observations.

found in 5 male mice only, which received 100 mg. per kg. of gallium per week and 0.3 mg. of methylcholanthrene and survived for 4 to 7 months. Four of these 5 mice had squamous cell carcinomas of the forestomach. Severe anemia, with hemoglobin readings averaging 7 gm. and erythrocyte counts of 3.8 to 7 millions, and a leukopenia with counts averaging 5,100 cells per cc. in 4 of these mice, can be ascribed to the effects of the neoplasm. No cause of anemia and leukopenia was found in the fifth mouse. In mice which were 16 months old or older, the lymph nodes showed plasma cell hyperplasia and moderate reduction in lymphocytes, while the spleen showed slight atrophy and an increase in hemosiderin deposits. Changes such as these are not unusual for mice of this age and may have been unrelated to treatment.

Lesions of the Forestomach

Forty-six (71 per cent) of the mice receiving both gallium and methylcholanthrene had lesions of the mucosa of the forestomach at necropsy. No lesions were found in the mice that did not receive methylcholanthrene (group F). These lesions in the forestomach ranged from dyskeratosis, acanthosis, and hyperkeratosis to grossly visible papillomas and squamous cell carcinomas that in many cases almost filled the lumen of the stomach.

These lesions are arranged into three groups in sequential order: (1) dyskeratosis, acanthosis, and hyperkeratosis; (2) papillomas; and (3) squamous cell carcinomas (Table II). As a general rule, mice with papillomas had some areas with abnormal keratinization, and animals with squamous cell carcinoma of the forestomach showed some areas with papillomas and some with disturbances in keratin formation. Squamous cell carcinomas were found in 34 per cent, papillomas in 17 per cent, and dyskeratosis in 20 per cent of the mice receiving methylcholanthrene. Implants of squamous cell carcinoma were found on the peritoneum in some cases. These lesions were similar to those observed in the rat by Mulay and Firminger[11] and in mice by Lorenz and Stewart[12] and Saxén et al.[13] They were described in detail by Stewart and Lorenz.[14]

Lesions of the Glandular Stomach

Severe, extensive, and specific changes occurred in the gastric glandular mucosa of animals treated with gallium and methylcholanthrene. The basal portions of the gastric glands from the limiting ridge to the pylorus showed degenerative changes. The parietal cells were affected to a greater degree than the chief cells. There was edema

of the gastric mucosa (Figs. 1 and 2), which was restricted to the basal portions of the gland, and which affected nearly every gland. The surface epithelium and the mucous neck glands apparently were unaltered. The chief and parietal cells, however, frequently were absent, or showed extensive degenerative changes, and occasionally were replaced by multinucleated giant cells or regenerating parietal cells.

The changes ranged from chromatolysis of a mild degree in several parietal cells of a particular gland to extensive and severe necrosis with pyknosis, karyorrhexis, extrusion of nuclei, and cytoplasmic lysis. With the most extensive change, almost all cells of the base of a gland were lysed, leaving occasional "ghost" cells, the stroma, and a rare viable cell (Figs. 3 and 4). Regenerative changes were present, and were most notable in the parietal cells (Fig. 3), the cytoplasm and nuclei of which were hyperchromatic. Occasionally, multinucleated cells were observed composed of apparently fused, immature, regenerating parietal cells. The changes observed in the glandular mucosa of mice treated with gallium salts alone (group F) were essentially the same as those described for the other groups.

Focal areas of adenomatous gastritis were observed in the glandular stomachs of 6 mice. This lesion was characterized by atypical and dilated glands as well as heterotopic mucosal glands in the submucosa. Often these glands were deep in the mucosa and filled with débris, fibrin, and leukocytes. The cells of the gland were often tall columnar, resembling mucous neck gland cells, but chief and parietal elements also were present. Aside from hyperchromatism, atypical structure, and location, the glands did not differ from the normal gastric mucosa. In all instances an inflammatory reaction of a mild degree, made evident by granulocytes and lymphocytes, was present in the submucosa about heterotopic glands. Often the heterotopic glands were surrounded by delicate connective tissue which was interpreted as being an accompanying stroma. Hare and Stewart[15] described the spontaneous occurrence of a similar lesion in dba mice.

Duodenal Lesions

Focal zones of mucosal alteration were observed in the duodenum and at the gastroduodenal junction in 5 of 11 animals examined. These lesions varied from atrophy to hyperplastic proliferation and the formation of adenomatous polyps (Figs. 5 and 6). Often these three processes co-existed in a well developed or extensive lesion. In these instances the mucosa of the duodenum adjacent to a polyp and

proximal to the stomach was atrophic, while the mucosa distal to the stomach was hyperplastic. In the atrophic areas, villi had almost completely disappeared. There was no change in the submucosa, or in the muscularis. The adenomatous polypoid lesions were sessile, and were located usually in the first or second portion of the duodenum. The superficial mucosa in the proliferative area often was hyperplastic. Frequently, inflammatory cells were absent in the edematous tips of the villi. In some places mucosa extended into the submucosa, forming a diverticulum compressing the glands of Brunner. There were no changes suggestive of malignant transformation in these polyps.

The hyperplastic zones often were distal to the polyp and manifested by elongation and thickening of the villi and hyperchromatism of the mucosal cells. These areas were small and blended gradually into the normal mucosa. The spontaneous occurrence of a similar lesion was described by Hare and Stewart[15] in dba mice.

The Renal Lesion

The principal lesion in the kidney was nephrosis, manifested as necrosis of the epithelium of the proximal convoluted tubules. This lesion was found in 44 per cent of the mice. The mildest change was hydropic degeneration of the epithelial cells, which was often of such a degree that the lumen of the tubule could not be identified (Fig. 7). Granularity and an intense eosinophilic staining reaction of the cytoplasm were present in most of the epithelial cells of the proximal convoluted tubules. In all cases, vacuolization, chromatolysis, karyorrhexis, and karyolysis were present either in widespread or focal zones in the cortex. "Ghosts" of the exfoliated epithelial cells filled the lumina of the proximal convoluted tubules and appeared as faintly outlined cells. These appeared to coalesce in the distal convoluted and collecting tubules to form large, hyaline, acidophilic casts resembling amorphous protein material. However, there was no evidence of obstruction of the tubules by these casts. Flattened cells with faintly basophilic cytoplasm, hyperchromatic nuclei, and increased mitotic activity were present in the epithelium of the proximal convoluted tubules. This was interpreted as regeneration. In one animal, calcification of the necrotic epithelium was seen.

Damage to the glomerulus was seen occasionally. When it occurred, it was evidenced by proliferation of the endothelial cells, or as indistinct hyaline material in the capillary tuft. Rarely, the outer layer of Bowman's capsule was irregularly and slightly thickened. In a few instances there were abnormally numerous cortical cysts. A mild in-

flammatory reaction was present in the interstitial tissues, but this was not universal.

These alterations were present in a much reduced form as long as 8 months after the gallium treatment was discontinued. In such animals, the proximal convoluted tubules showed only desquamation into the lumina, minimal hyperchromasia, and vacuolization. Casts were present in the distal convoluted tubules, and there was evidence of calcification or interstitial inflammation.

DISCUSSION

Lesions observed in the forestomach of mice in these experiments are similar to those produced by White and Stewart[16] and by Lorenz and Stewart[12,17,18] in many strains of mice, including C57Br, by feeding them methylcholanthrene. These investigators used an oil emulsion of the carcinogen, and their animals ingested 80 to 240 mg. of the hydrocarbon during the course of the experiment. They observed intestinal carcinoma in their animals as well as squamous cell carcinoma of the forestomach. In the present study, although 33.8 per cent of the animals developed squamous cell carcinoma of the forestomach, no intestinal carcinoma was observed. This difference may be due either to the vehicle used (polyethylene glycol) or to the quantity of the hydrocarbon ingested (6 to 30 mg.), or to both. As mice receiving gallium alone did not show lesions of the forestomach, it is believed that it did not contribute toward the production of this lesion.

Lesions of the mucosa of the glandular stomach were produced in all animals whether they received gallium alone or with methylcholanthrene. These lesions were first observed by Eyestone[9] in mice which received gallium citrate alone. Thus gallium was responsible for the lesions of the gastric mucosa. The dosage of gallium used, 40 to 100 mg. per kg. per week, did not make a significant difference in the severity of the lesion. However, the life span of the animals receiving a high dosage of gallium was shortened.

The nephrotic lesions of the kidney caused by injections of gallium citrate were very similar to renal damage caused in man by ingestion of salts of heavy metals like mercuric chloride and potassium dichromate. Similar lesions were observed in mice receiving gallium alone.[9] These lesions are similar to those found after the tragic use of diethylene glycol as a vehicle for sulfanilamide. However, mice in group A, without any polyethylene glycol, developed the same lesion with equal frequency (Table II). It is remarkable that a mild renal lesion persisted in these mice for as long as 8 months after the injection of gallium citrate was

discontinued. Spontaneous hydronephrosis has been reported in C57Br mice[19] but not spontaneous nephrotic lesions. Therefore, it is concluded that gallium was responsible for this lesion.

Since adenomatous gastritis and duodenal lesions have been found in untreated old dba mice[15] and since these lesions were found only in a few animals (5 each) about 16 months or older, it is assumed that these lesions were spontaneous in the C57Br mice. However, the toxicity of gallium cannot be ruled out as a contributory cause.

SUMMARY

Mice of the C57Br strain receiving doses of gallium of 40, 60, 80, and 100 mg. per kg. of body weight per week for 22 to 28 weeks and receiving 0.27 mg. of methylcholanthrene per week for the same period, developed lesions in the glandular stomach, forestomach, kidney, and duodenum. Of these lesions, hyperkeratosis, papillomas, and squamous cell carcinoma of the forestomach were induced by methylcholanthrene. Lesions of the glandular stomach, namely, destruction of parietal and chief cells, were induced by gallium. Gallium may have contributed to the renal damage. Lesions of the duodenum probably were spontaneous.

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[*Illustrations follow*]
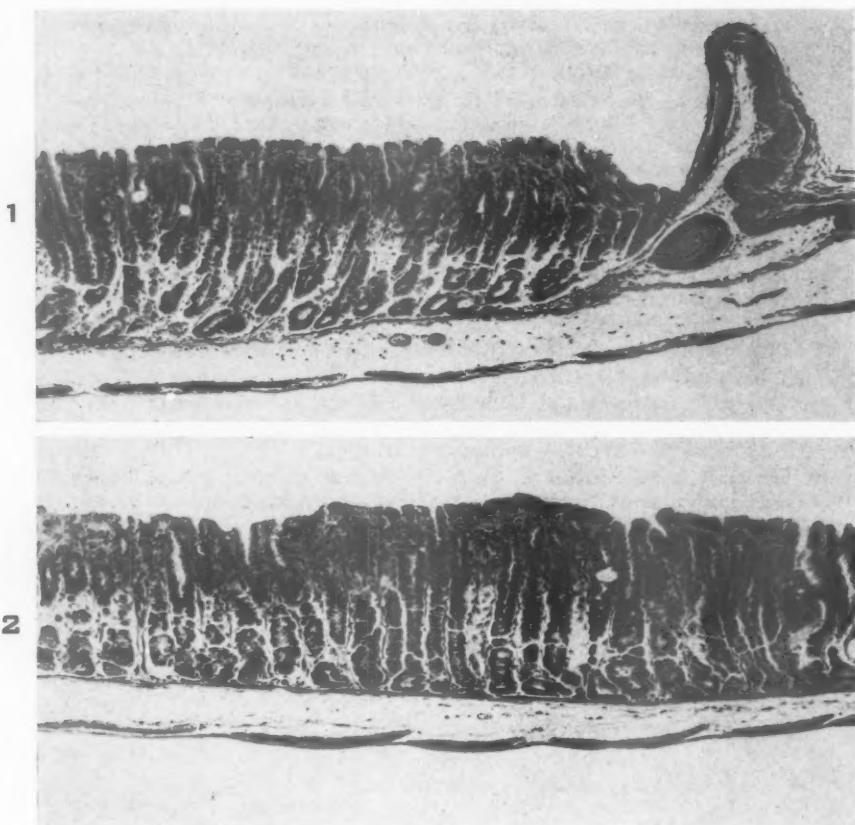
LEGENDS FOR FIGURES

All sections were stained with hematoxylin and eosin.

FIG. 1. Area of glandular stomach adjacent to limiting ridge, showing moderate to severe destruction of parietal cells in almost all glands. $\times$ 95.

FIG. 2. Glandular stomach showing severe degenerative change involving parietal cells in some glands, while all cellular elements in other glands are well preserved. The surface epithelium is normal and the mucous neck layer is intact despite severe damage to cells. $\times$ 105.

FIG. 3. Destruction of parietal and chief cells of the base of a gastric gland with preservation of mucous neck cells. Multinucleated regenerating parietal cells are present. $\times$ 400.

FIG. 4. Glandular gastric epithelium showing destruction of both parietal and chief cells in a single gland. Adjacent glands are spared. $\times$ 360.
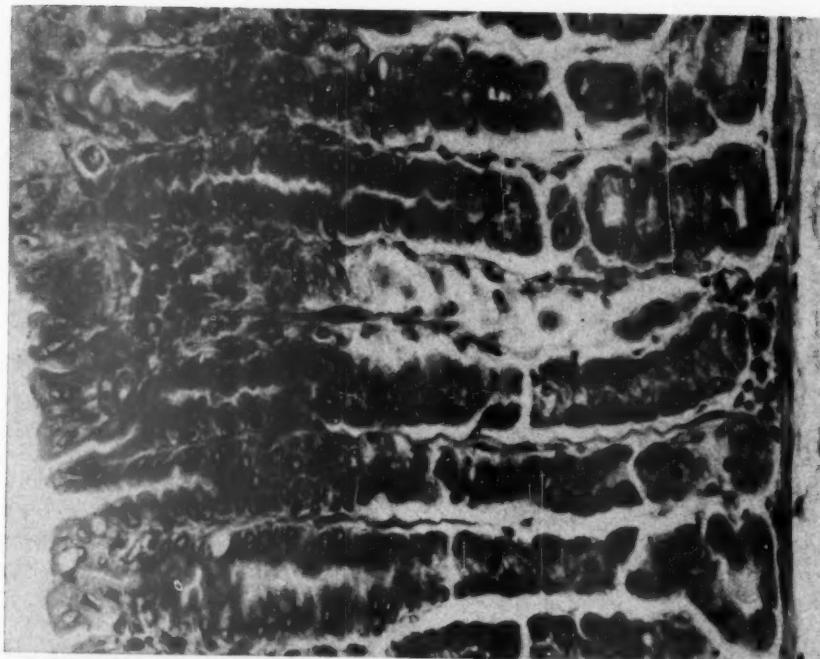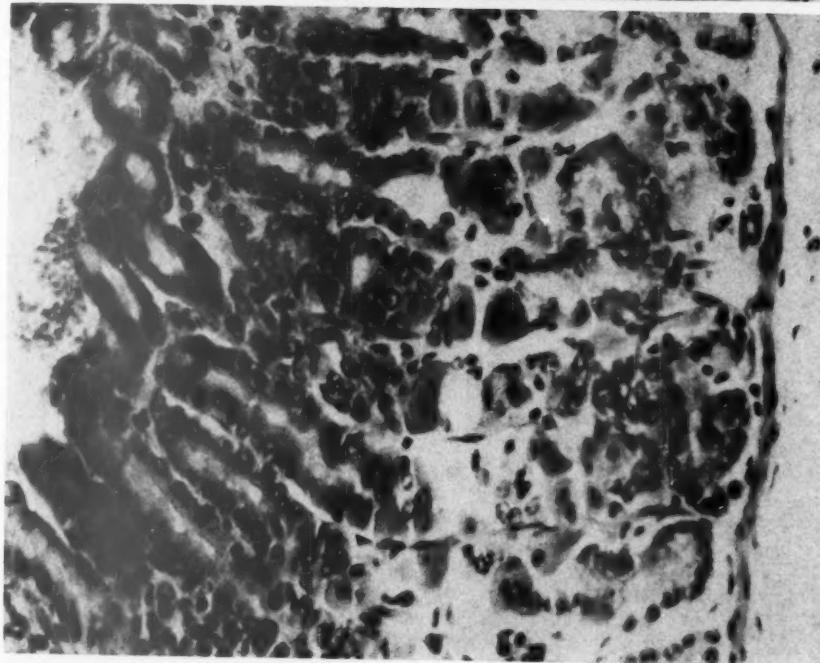
FIG. 5. A duodenal polyp with atrophy of the epithelium proximal to the lesion and hyperplasia of the glands of Brunner in a zone proximal to and underlying the polyp. × 16.

FIG. 6. Duodenal lesion showing epithelial atrophy of the mucosa, thickening of the layer of Brunner's glands, and downgrowth of surface epithelium into the submucosa. × 35.

FIG. 7. Renal cortex showing tubular casts, and hydropic degeneration of cells of the proximal convoluted tubules. × 360.
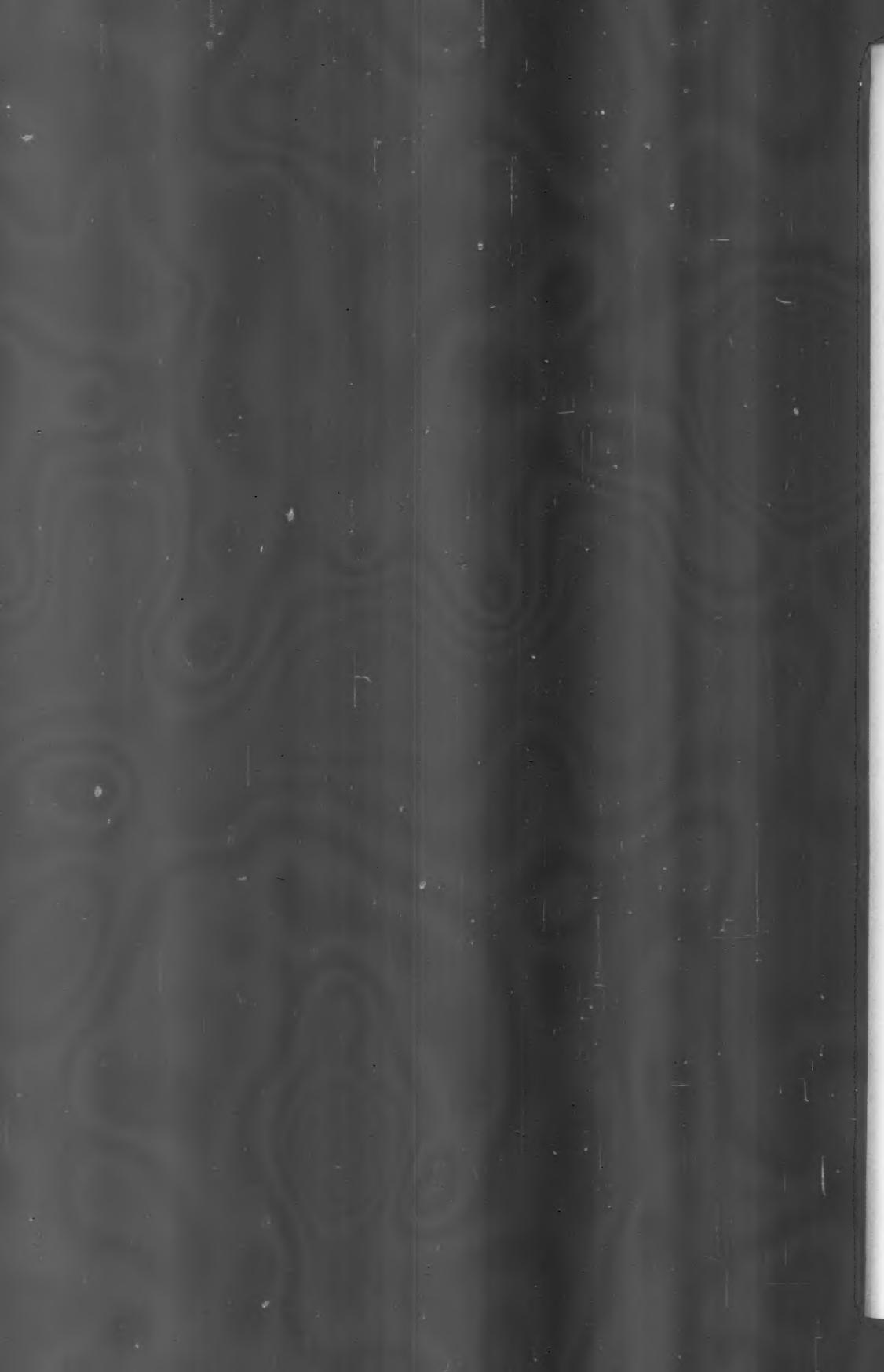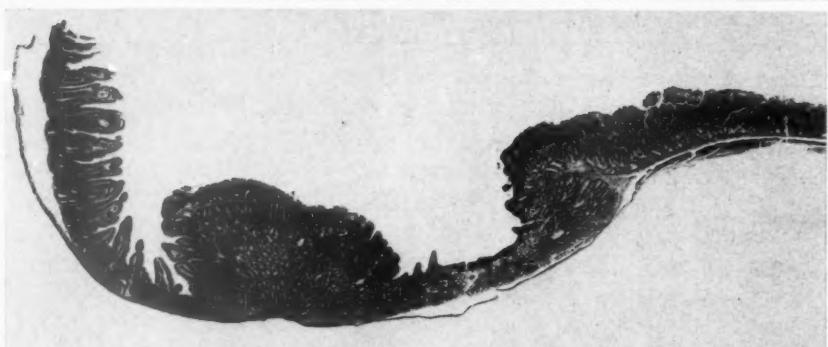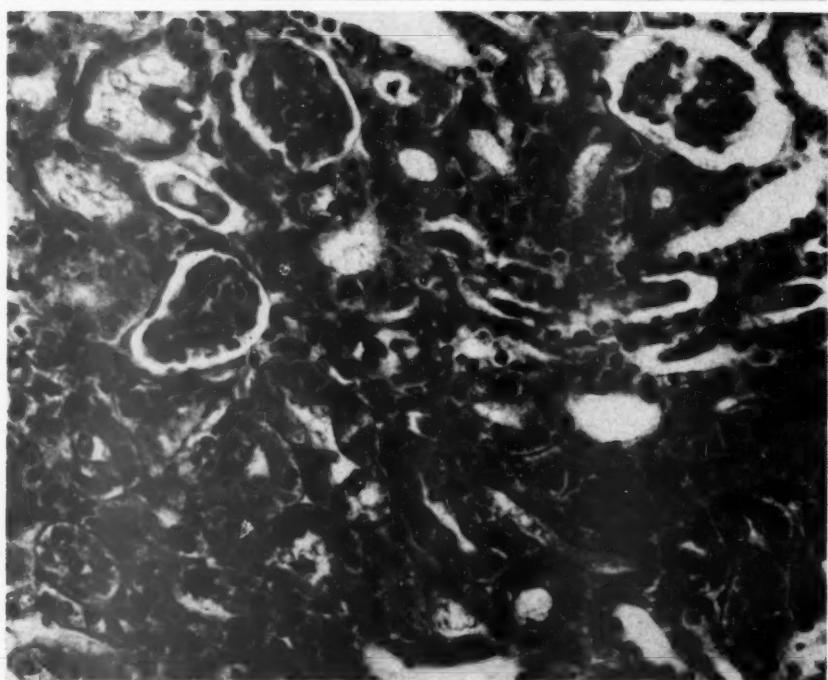
THE EFFECT OF TRAUMATIC INJURY ON THE BRAIN
OF VITAMIN-C-DEFICIENT GUINEA-PIGS *

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The rôle of vitamin C in the formation of ground substances and connective tissues in various organs has been the subject of a number of investigations.[1-3] Although the exact mechanism of its action is not known, histologic and biochemical studies indicate that in scurvy there is either a lack of production of the ground substance or failure in the polymerization necessary to the formation of extracellular connective tissue.[4-7]

The relationship of vitamin C deficiency to wound healing is well known. Experimentally, it has been shown that wounds of scorbutic animals fail to heal normally because of faulty formation of collagen and lack of proliferation of capillaries.[1,3] As far as we are aware, the only publication on the effect of vitamin C deficiency on the nervous system of experimental animals is that of Meyer and McCormick.[8] In guinea-pigs maintained on a scorbutic diet from 40 to 135 days, they found perivascular petechiae in the brain with a few in the spinal cord, posterior root ganglia, and nerve trunks. The only parenchymal changes were perivascular sponginess adjacent to the hemorrhages and possible regressive changes in posterior root ganglia. From the account of the diet employed and from the atypical pathologic changes encountered in peripheral nerves, adrenal cortex, and other viscera, particularly the liver, it seems likely that these animals had multiple nutritional deficiencies.

The object of the investigation reported here was to determine whether changes develop in the brain in experimental scurvy and to what extent vitamin C influences the healing of traumatic cerebral lesions.

METHODS

In the first experiment, 9 guinea-pigs, 3 to 4 weeks old and weighing about 250 gm., were given a vitamin-C-free Rockland guinea-pig diet supplemented with adequate amounts of yeast and vitamins A, D, and E. Details of this diet have been reported previously.[9] In order

to maintain the animals on the deficient diet for an adequate period (beyond 4 weeks), a small maintenance dose of vitamin C, 0.2 mg. per day, was administered. A total of 13 control animals were fed the same diet plus vitamin C at a level of 2.0 mg. per day, which is the normal daily requirement of young guinea-pigs. Animals from both groups were sacrificed by nembutal anesthesia at the end of 3, 6, 8, 12, and 16 weeks. The brains were fixed in 10 per cent neutral formalin, sectioned at 6 μ, and stained by hematoxylin and eosin.

In the second experiment, the response to traumatic injury of the brain was studied in 8 deficient and 10 control animals. After an initial period of 10 days on the deficient or control diet, a burr-hole was made in the skull through which cerebral trauma was produced by deep puncture of the parietal or occipital part of the brain with an 18 gauge needle. Sterile precautions were observed. One or 2 animals from each group were sacrificed under nembutal anesthesia at the end of 3, 5, 10, 15, and 22 days following brain puncture. The brains were fixed and sectioned as in the first experiment, and were stained by hematoxylin and eosin, and Masson's trichrome, periodic acid-Schiff's, cresyl violet, the Gomori reticulum, and Holzer glial fibril techniques. Frozen sections were stained for fat by oil-red-O. Evaluation of the response to the traumatic injury in experimental and control animals was based on the nature of the cellular reaction at corresponding distances from the needle track.

RESULTS

At the time of sacrifice all experimental animals presented gross and microscopic evidence of scurvy, particularly in the bones, joints, and periarticular tissues. The severity of the lesions was directly related to the duration of the deficient diet.

In the second experiment the cellular response to injury differed in the two sets of animals. By the end of the third day, gitter cells were seen in the necrotized parenchyma of the needle track in all animals. By the fifth day the wall of the track of the control animals was laden with gitter cells, and rows of elongated cells with the characteristics of fibroblasts ran parallel to the track (Fig. 1). These alignments suggested early attempts at capillary formation. Pre-existent vessels in the wall of the track showed moderate proliferation of endothelial and adventitial cells. In the scorbutic animals at the 5-day stage, gitter cells had formed, but they were fewer than in the control animal and there was no suggestion of capillary formation (Fig. 2). The same was true at the 10-day and 15-day stages as compared to the control animals.

In the control animals, progressive removal of the products of hemorrhage and necrosis occurred so that by the 22nd day only a small collection of pigment-laden macrophages remained at the site of the injury. At that time there was an abundant cellular response in a wide parenchymal zone adjacent to the track. The majority of the proliferated cells were astrocytes, a few were oligodendrocytes, and a fair number were unidentified. The Holzer glial fibril stain was negative. The vessels closely adjacent to the track showed pronounced enlargement with moderate proliferation of endothelial and adventitial cells. As to the meninges, they showed progressive reactive changes from about the third day onward. Meningeal fibroblasts steadily increased in number and underwent differentiation, as judged by cellular contents and configuration. By the 22nd day, fibroblasts had migrated from the edge of the traumatized area into the mouth of the track. Whether in syncytial arrangement or lying free, the fibroblasts had concave borders and granule-free perikaryon. Some fibroblasts were being aligned to form capillaries, and occasional newly formed capillaries contained erythrocytes (Figs. 3 and 4).

In the scorbutic animals, the neuroglial response was approximately the same as in the control animals. As to the vascular reaction, the changes in the large vessels were similar to those in the control animals, but somewhat less pronounced. In an animal surviving 15 days the reaction in the wall of the track was rather slight and there was no meningeal fibroblastic response whatsoever. At the 22-day period, the meningeal reaction was significantly less pronounced than in the control (Fig. 5). Proliferated fibroblasts were seen but were much fewer than in the control animals. Syncytial arrangement of the fibroblasts was noted here and there, but was poorly developed. In most regions the cells were collected in groups reminiscent of pavement epithelium. Not only was the syncytium poorly differentiated, but the individual cells also appeared unlike those of the controls in that the perikaryon was granular and cytoplasmic processes had failed to develop (Fig. 6).

DISCUSSION

These results confirm previous observations of Meyer and McCormick[8] that changes in the parenchyma of the brain are lacking in vitamin C deficiency. On the other hand, we were unable to confirm the presence of petechiae in the brain reported by these workers. This discrepancy may be attributed to the small maintenance dose of vitamin C used in our experiments. Lack of healing of the brain wound in the vitamin-C-deficient animals was obvious, particularly at the

mouth of the wound in the region of the meninges. The differences in the reactions in experimental and control animals at later stages were regarded as significant, inasmuch as at later stages no differences in incidental factors, such as the number of erythrocytes or of hemosiderin-laden macrophages at the site of puncture, were detected.

This lack of healing of traumatic lesions in the brain is not appreciably different from that in other organs of the body of vitamin-C-deficient animals in which lack of differentiation of mesenchymal elements and formation of granulation tissue are characteristic.[2,7,10] By contrast, no differences between experimental and control animals were noted in the reaction of the neuroglia, which is in line with the observations that in vitamin C deficiency the parenchymal cells are not appreciably affected.[11,12] It is of interest that proliferation and activity of phagocytic cells also were not impaired in the scorbutic animals although these elements are of recognized mesenchymal origin. This confirms observations of other investigators in the field of scurvy, and in that of chronic inanition as well.[9,13]

SUMMARY

In scorbutic guinea-pigs with uninjured brains, no changes in the brain were observed. On the other hand, in scorbutic guinea-pigs subjected to puncture wound of the brain, the proliferative and reparative reactions of mesenchymal elements in the traumatized meninges and parenchyma were much less pronounced than in the control animals. Thus, the response to injury to the brain in scurvy is not essentially different from that seen in the body.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

All sections were stained by hematoxylin and eosin.

Fig. 1. Control animal (Armed Forces Institute of Pathology no. 272887) 5 days after production of puncture wound. Numerous robust gitter cells may be seen in the wall of the track. There is alignment of narrow cells suggesting capillary formation. × 260.

Fig. 2. Scorbutic animal (A.F.I.P. no. 272890) 5 days after production of puncture wound. Numerous gitter cells are present. The brain substance shown in the lower part of the photomicrograph has undergone coagulation necrosis, to which there has been no glial response. × 260.

Fig. 3. Control animal (A.F.I.P. no. 300831) 22 days after production of puncture wound. Proliferated meningeal fibroblasts are numerous. × 75.
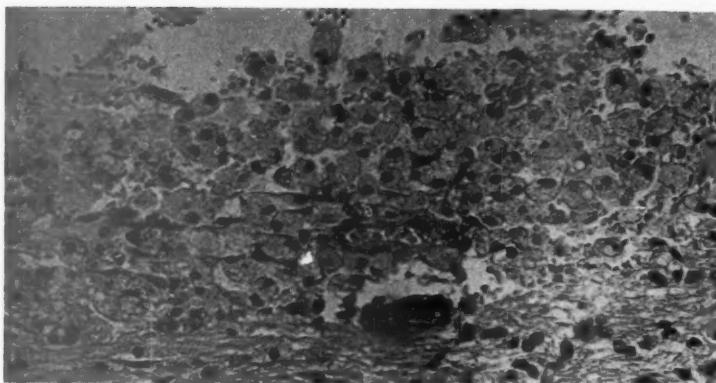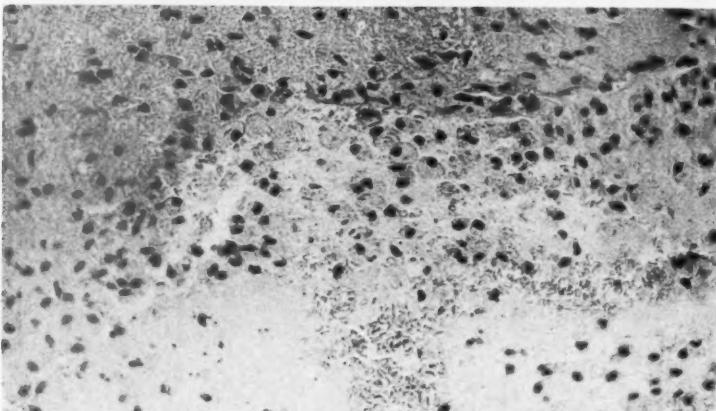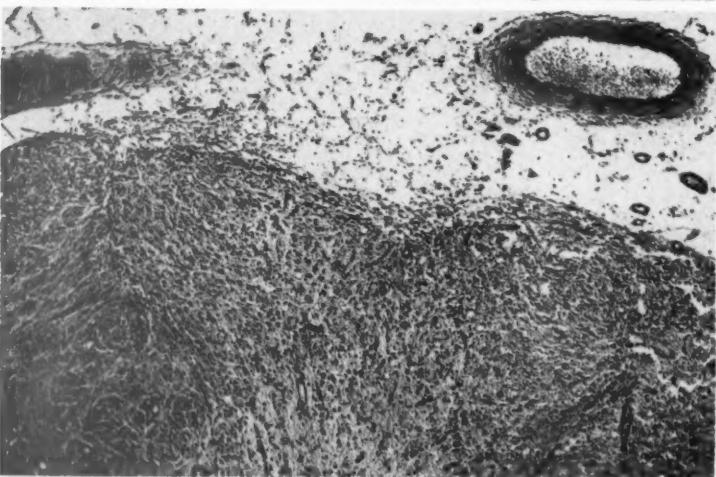
FIG. 4. High-power view of field shown in Figure 3. Numerous meningeal fibroblasts
are visible. ✕ 600.

FIG. 5. Scorbutic animal (A.F.I.P. no. 300830) 22 days after production of puncture
wound. There is virtually no meningeal reaction. ✕ 75.

FIG. 6. Same case as that illustrated in Figure 5. Proliferated meningeal cells are
poorly differentiated. ✕ 600.
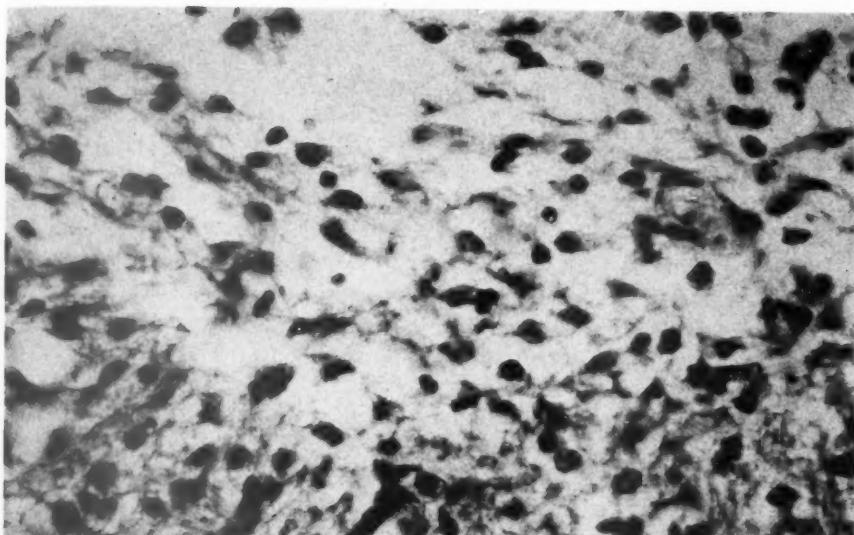
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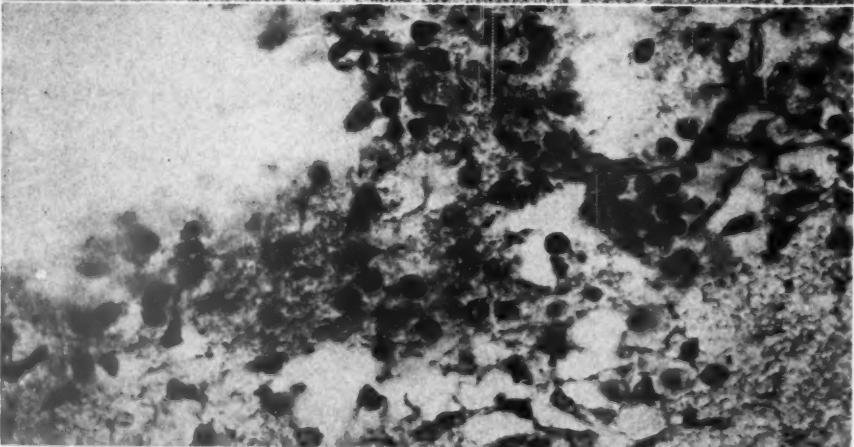
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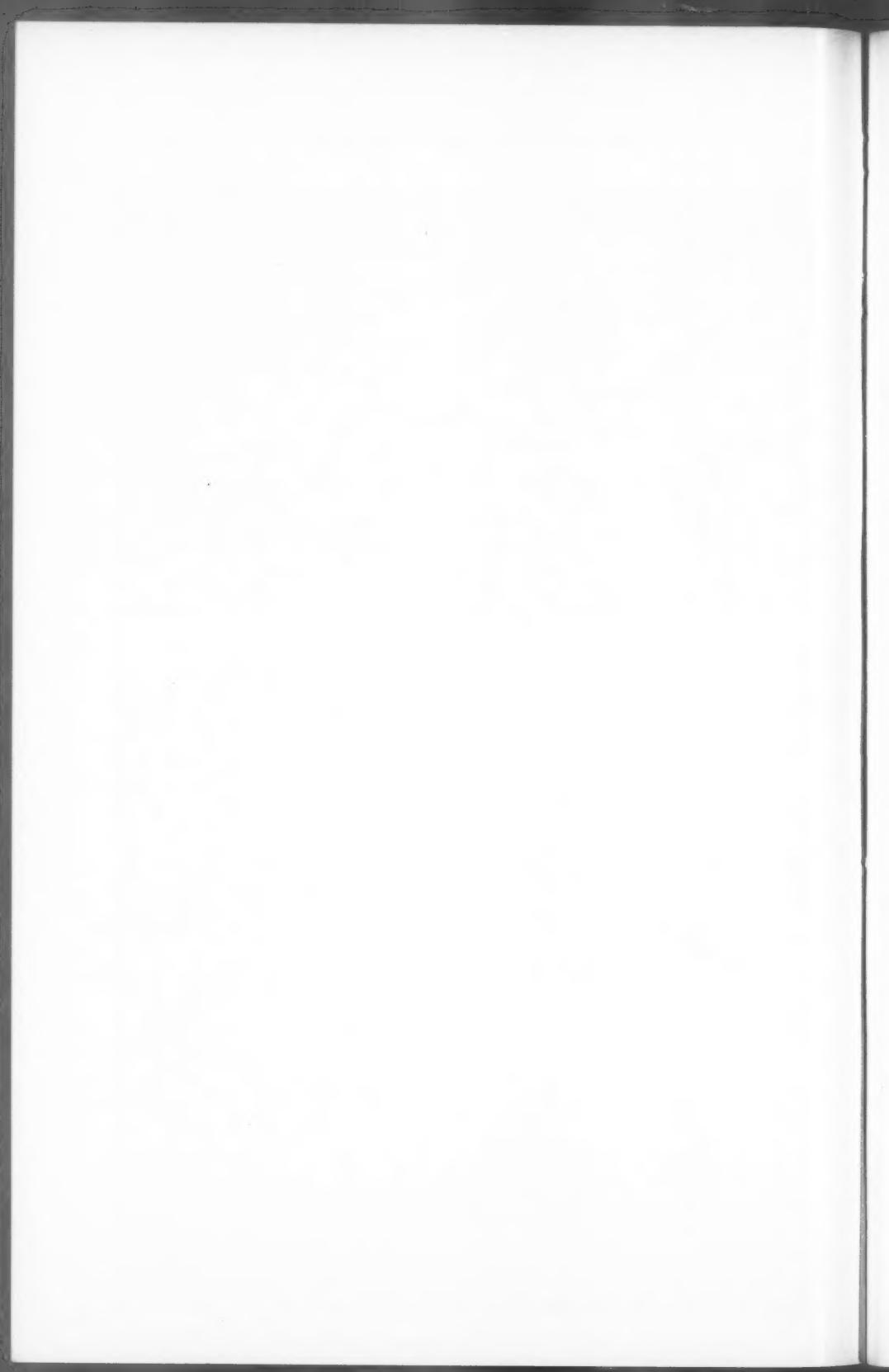
PULMONARY DISEASE IN RATS

A SURVEY WITH COMMENTS ON "CHRONIC MURINE PNEUMONIA" *

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The albino rat is less susceptible to acute pulmonary infections, both natural and induced, than other rodents used for laboratory work, and therefore is not commonly used in experimental bacteriologic and virus studies. If ideal conditions of breeding are established, including strict control over general hygiene, nutrition, and disease, rats can be bred which show remarkable freedom from epizootic infections, and in which acute pneumonia does not play a prominent rôle. Acute pulmonary infections in rats, caused by Pasteurella, *Brucella bronchiseptica*, and pneumococci, have occurred with epizootic spread and high mortality, but such outbreaks are uncommon. Nelson (who has conducted the most extensive investigations on pulmonary disease in rats in the United States) mentioned,[1] in 1953, that in 20 years he has observed only one acute epizootic outbreak. One of us (J.R.M.I.) had a similar experience while in charge of an "Animal Breeding Farm" in England (1940–46), in which a self-contained colony of rats was established, productive of more than 20,000 rats a year. However, in our own laboratories in recent years, two acute epizootics of pneumonia have occurred, one caused by type II pneumococci, and the other, mainly by Pasteurella.

In all laboratories in which large numbers of rats are used experimentally, morbidity (less importantly, mortality) due to chronic pulmonary disease is a perennial problem. It may be more striking in commercially obtained rats, for the animals are then of unknown clinical history, and reared under conditions not defined by experimental users. Although the nature of the disease has long been known, until recently it was a confused complex. Pathologists who examine many rats are familiar with the common macroscopic finding of involvement of a part or whole of a lobe, or of several lobes of the lungs by areas of induration and bronchiectasis. Most diseased rats exhibit few, or no, clinical signs of respiratory disease, although in advanced cases there may be snuffles, torpor, rough hair, and dyspnea. These signs are recognized as being unreliable by those dealing with rats, and the lesions are thus usually dismissed at necropsy as incidental findings or cursorily noted as of little moment. This is particularly

true in experiments not specifically concerned with the lungs, or on occasions when necropsies are done by those with limited experience. In view of the incidence of pulmonary disease in rats, it is remarkable that it is so rarely mentioned as a complicating factor considering the many experiments in which rats are used. We give our own data later, but a few published figures on the incidence can be quoted[1-5] (Table I), and these must be given serious consideration.

TABLE I
Incidence of Chronic Murine Pneumonia

Source	No. of rats examined	Percentage affected
Passey *et al.* (U.K.)[2]	251	51
Cruickshank (U.K.)[3]	200	43.5
Klieneberger and Steabben (U.K.)[21]	268	40
Wilens and Sproul (U.S.A.)[5]	487 (all over 700 days of age)	75
Ratcliffe (U.S.A.)[4]	Not given	75

Whenever the bulk of work with rats concerns chronic toxicity, or toxicopathologic, studies with particular reference to pulmonary damage caused by chemical compounds, the incidence and severity of natural disease become of conspicuous significance. From this standpoint, thousands of rats have been used by our laboratories in a multitude of experiments, and it is apparent to us that the problem of chronic pulmonary disease has been mostly ignored. The opening sentence of a paper by Cruickshank[3] (1948) stated: "In the course of some tentative experimental work on lung diseases it was found that the stock laboratory rats employed suffered from a spontaneous disease of the lungs which made the experiments *useless*." His experience in England and his opinion are not unique. In work of our own type, the difficulty of determining changes caused by a toxic inhalant material from those resulting from, or complicated by, natural disease sometimes has been insuperable. We have thus raised the issue that rats might advisedly be discarded for work involving toxic inhalants, unless the animals are derived from a colony in which chronic pulmonary disease does not exist; and Nelson[6] has shown that it can be eradicated. Unless this is achieved, a more suitable, small, laboratory rodent might be the hamster, which is as fecund as the rat, and seems to be relatively free from chronic pulmonary disease, although susceptible to acute infections.

Survey of Chronic Pulmonary Disease in Rats at Chemical
Corps Medical Laboratories

METHODS

Many observations have been accumulated from the large numbers
of rats (a total of 3,646 in the years 1953 and 1954) that are necrop-
sied in connection with toxicopathologic experiments. For comparative
purposes, however, selected groups of rats (totaling 433) were killed
for special examination of the lungs. These were rats which to all
appearances were clinically healthy, the younger of which would nor-
mally have been issued to investigators. Apart from one group of aged
rats, all animals were bred in the Medical Laboratories colony. After
sacrifice (usually with ether) the lungs were examined in a collapsed
state, or after ligating the trachea before the chest was opened. Sec-
tions were made of the lungs of all animals and, along with the hun-
dreds of others from past years, these sections form the basis for our
later definitions of the lesions of this disease. All sections were stained
with hematoxylin and eosin, and in selected instances with Wilder's
reticulum stain, Weigert's elastica, and phosphotungstic acid-hema-
toxylin. Bacteriologic cultures were made at various times, but not as
a routine procedure.

FINDINGS *

Based on gross observations and histologic examination, a general
assessment was made of lesions in the lungs related to different age
groups, and the results are given in Table II.

Because of the variation in the numbers of animals in the different
groups, the different times of the year when the rats were killed, differ-
ences in husbandry, and other variables, exact comparisons are difficult.

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* *Remarks on Some Normal Anatomical Features of the Rat Lung.* In the rat (also the
hamster and mouse) the right lung has three main lobes (apical, cardiac, azygos, and a small
intermediate caudal one) exceeding in anatomical mass the left lung, which is a single lobe.
There are no fundamental differences between rodents in the descending arborization of the
respiratory tree. The right main bronchus is short and abuts from the trachea at a near right
angle, while the left main bronchus descends obliquely into the hilus of the left lung; the
right main bronchus immediately gives off a shorter trunk for the most cranial (apical) lobe
of the lung—the so-called epi-arterial branch. Cartilaginous plates are found in the walls
of secondary bronchi of rabbits and guinea-pigs but, in the rat, mouse, and hamster, carti-
lage fades from the walls of the bronchi as soon as they enter the lungs. The adventitia of
the pulmonary veins in the lungs of the rat and mouse shows a peculiar normal feature of
striated muscle fibers contiguous with those of the heart.[7-9] These cardiac muscle fibers are
present in the larger intrapulmonary branches, but may be seen in small veins (Fig. 1). In
rabbits and guinea-pigs such fibers are reputed to surround only the short extrapulmonary
veins. This might have some physiologic significance, but has been given little attention,
other than in the German literature on histology. It might, however, be of some significance
pathologically, in the sense that infections with a predilection for heart muscle could spread
by contiguity to the lungs. (See work by Pappenheimer and Daniels[10] on a transmissible
"rickettsial" disease of mice causing myocarditis.)

Some general conclusions can be drawn. The figures are in agreement
with the experience of other workers[1-5] on the high incidence of a dis-
ease which may not kill, or even cause any apparent disturbance in
health, and which becomes more frequent with advancing age. It is
apparent that the macroscopic appearances alone may not lead to the
detection of this disease. Of 433 rats of all ages, 216 showed normally
pink crepitant lungs (69 of these were 3 weeks or less old).

Likewise, the number considered normal histologically would be
reduced if a more critical examination were adopted. For example, if
microscopic collections of lymphoid tissue were considered pathologic,

TABLE II
Range of Normality in Lungs of Rats

| Group | Age | Numbers | Gross | | Microscopic |
			Normal	Pneumonia	"Excess" lymphoid tissue*
A	Under 3 weeks	19	19	0	2
B	3 weeks	50	50	0	50
C	2 months	39	18	21	18
D	3-4 months	7	4	3	4
E	4 months (diet exp.)	95	11	84	11
F	5-6 months	22	14	8	10
G	7-12 months	25	21	4	20
H	12-15 months	103	35	68	28
I	15-17 months	23	12	11	12
J	Over 12 months (commercial)	50	32	18	24
	Totals	433	216	217	179

* In lungs which were grossly normal. Grossly normal lungs which were devoid of "excess"
lymphoid tissue were found in only 37 rats.

the number of so-called normal lungs would be smaller. Lymphoid
tissue in any amount is unusual (yet occasionally seen) in suckling
rats, but microscopic aggregations were observed in all of the 50 newly
weaned (21 days) rats. In rats with chronic disease there is always
marked lymphoid participation. This lymphoid tissue involves the
walls of the bronchi, and is found in perivascular locations, particularly
about arterioles and venules. There is proportionately less involvement
of the bronchioles. Involvement of the bronchial tree varies in degree
from small to massive aggregations of lymphocytes, infiltrating the
deeper parts of the bronchial mucosa and pushing aside and obliterat-
ing all structures, with cleavage and disappearance of the normal elas-
tic and muscular fibers (Fig. 2). Examination of the stained sections,

simply by the naked eye, shows the bronchial arborizations outlined by broad, blue-stained, interrupted sleeves (Fig. 3). This lymphoid nodular lesion, which might well be referred to as a follicular bronchitis, was present in very many rats without an associated bronchopneumonia. Hence, we can understand the views previously expressed about its rôle in the production of bronchiectasis (e.g., Passey *et al.*[2] and Cruickshank[3]). These data indicate the extremely high incidence of all stages of a chronic disease complex, which may be found in almost any group of our laboratory rats and in other colonies as well.*

However, the criteria which were used to compose Table II serve only as a rough separation of normality from abnormality. If a more strict yardstick is utilized, we find that the lungs of most adult rats are significantly abnormal. (There was no difference found by us between our own rats and those obtained commercially.) If comparisons are to be made by others between our figures and their own, they will be largely invalid unless comparable detailed histologic examinations are made.

An illustration of the importance of this conclusion can be found in some toxicity experiments made in our own laboratories. Previously, there had been preliminary reports that chronic bronchitis ensued after protracted inhalation exposure to diborane (B_2H_6) in low concentrations. It was manifest that such a conclusion would be difficult to substantiate, inasmuch as the "normal rat" has in most instances some degree of chronic bronchitis, and studies were made to clarify this. Four groups of 20 rats were selected according to age (5 weeks, 9 weeks, 6 months, and 1 year old). These groups were divided equally so that 10 animals served as untreated controls and 10 were exposed to diborane (6 ppm.), 4 hours per day, 5 days per week, for 6 weeks. At the end of this period all animals were sacrificed and the lungs subjected to detailed histologic examination. A sample of the protocol used in the critical histologic evaluation of the lungs is given in Table III, and needs little comment. Each feature was noted as present or absent, and, when applicable, graded from 1 to 4. We were unable to confirm the conclusion that chronic bronchitis was a toxic effect, and in only one respect was a predictable difference noted between treated and untreated rats. Foamy macrophages were found more frequently, and in greater numbers, in the alveolar spaces of animals exposed to diborane, a finding for which we have no explanation. It should be

* An examination, for instance, of the lungs of 50 male and female rats, reputed to be breeders over 15 months old, received from a well known rat colony in England, revealed an incidence of 11 with marked macroscopic lesions, and 28 with histologic lesions, including excess lymphoid tissue.

TABLE III

Schema for Evaluation of Histologic Changes in the Lungs

	1	2
Lymphoid infiltration, bronchial tree		
Mucosal		
Disruption of basement membrane		
Submucosal		
Bronchial musculature		
Follicle formation		
Limited to large bronchi		
Limited to small and medium bronchi		
Limited to bronchioles		
Extra-cartilaginous		
Perivascular lymphoid		
Large vessels		
Small vessels		
Thickening of alveolar walls		
Inflammatory		
Hyaline		
Patchy		
Diffuse		

	1	2
Alveolar spaces		
Acute exudate		
Chronic exudate		
Macrophages		
Patchy		
Diffuse		
Pleural thickening		
Fibrosis		
Focal		
Diffuse local		
Diffuse general		
Bronchial dilatation		
Bronchiolar dilatation		
Exudate in bronchi		
Destruction of bronchial epithelium		
Epithelial regeneration		
Epithelial hypertrophy		

noted, however, that such foamy macrophages often are found in the alveolar spaces (Fig. 4) either singly or in solid masses, infrequently containing a brownish pigment, in all age groups from suckling rats on, and in otherwise healthy animals.

COMMENTS ON "CHRONIC MURINE PNEUMONIA"
Macroscopic Appearances

The lesions may be discrete and affect a part or all of a lobe, or they may be disseminated. If discrete, the involved area is gray to red, indurated, and somewhat depressed. When a whole lobe is involved, it is shrunken, has a cobbled surface, and is rubbery. Disseminated foci generally are small, sharply circumscribed, reddish brown, millet-like masses (Fig. 5). These areas of induration cut easily and the exposed surfaces appear flat, dry, and homogeneous. There may be cyst-like spaces, which are actually dilated bronchi filled with mucoid or mucopurulent material. The lesions progress slowly, and may be of long duration before the lung is affected to the extent of causing respiratory incapacity, which in rats is still difficult to measure in a clinical sense. In late lesions, the affected lobe, or lobes, are markedly distorted by nodular masses which often appear as pinkish or pearl-gray protuberances. These marked bronchiectatic areas are filled with caseous débris (inspissated exudate), and superficially may resemble abscesses (Fig. 6). These latter lesions have not been particularly frequent in our own experience, but in our laboratories, rats used for experiment and necropsied are seldom old animals. It is noteworthy that pleural adhesions or empyema are relatively rare findings, an observation which will be commented upon later. It is rare, indeed, to find a chest cavity in the rat in which the entire thoracic contents are matted together. The severity of the morbid process is not necessarily reflected by severe clinical signs, and many observers have expressed astonishment at the extent of pulmonary disease in rats which appeared in good condition when alive. As the disease advances, however, unthriftiness may develop, with roughened hair, loss of weight, snuffles, and wheezing. There is no tendency for any particular lobe to be affected more frequently, as shown by the lobe involvement in 100 diseased rats (Table IV).

Microscopic Appearances

From the foregoing, it can be seen that much of the histologic picture can be accounted for by the variation in the type and degree of lymphoid infiltration. That this lesion begins very early in life is apparent from finding microscopic lymphoid collections in some suckling rats. This is almost a universal effect by the time of weaning. Involve-

ment of the bronchial tree is first noted in its proximal parts, but as the rat ages, the process extends to embrace the peripheral components. Bronchiolar disease at this stage can be said to be relatively mild. The lymphoid tissue implicates all layers of the bronchus and at its height is of a massive kind, in which formation of primary follicles always is found.

Perivascular collections of lymphocytes occur as early as peri-bronchial involvement, and there need be no quantitative correlation between the two. Perivascular infiltration is most conspicuous about small vessels—arterioles and venules — and frequently large cuffs and sleeves are found (Fig. 7). In younger rats, eosinophils are fairly numerous and pigment-laden phagocytes sometimes may be present.

TABLE IV

Involvement by Lobes in 100 Rats with Gross Lesions of Chronic Murine Pneumonia

Left lobe	25
Right cranial lobe	29
Right middle lobe	30
Right caudal lobe	31
Right intermediate lobe	26
Total number of lobes involved	141

Parenchymal involvement by lymphocytes tends to be focal at first, in the form of a patchy chronic interstitial pneumonitis (Fig. 8). Locally diffuse lesions soon make their appearance, sometimes a whole lobe showing inflammatory thickening of the alveolar septa. Inflammatory cells within the alveolar spaces usually are less conspicuous. The presence of foamy macrophages (sometimes with pigment) has been mentioned. The changes of bronchopneumonia seem to be relatively *infrequent*. Far more often a picture simulating bronchopneumonia is produced by a combination of atelectasis and interstitial pneumonia. It is true that focal abscesses are observed occasionally, but the correctness of the conclusion stated is suggested by the unique rarity of adhesions or empyema.

As the process advances with age, the picture becomes more complex. Dilatation of the bronchial tree in a segmental fashion and accumulation of secretions increase, and there is more and more atelectasis. Peribronchial fibrosis can be discerned readily, but what, at first glance, may appear to be extensive pulmonary fibrosis, may turn out to be marked atelectasis (Fig. 9). The bronchiectatic process may continue to a stage in which a lobe, or a whole lung, consists of multilocular spaces surrounded by collapsed and compacted parenchyma (Fig. 10). It is in the more advanced stages that bronchiolectasis may appear.

Many authors have commented upon the occurrence of squamous metaplasia of the bronchial epithelium, although it has not been common in our experience. It may be limited to a segment, or be widespread, even extending into alveolar ducts and alveoli, and its true nature should be recognized. Passey *et al.*[2] (and others) have pointed out that such metaplasia was mistaken for metastases by Fibiger[11] in his pioneer work on the relationship of a supposed gastric carcinoma to the parasite *Gongylonema neoplasticum*. This change, with keratinization, may be very prominent and has some superficial resemblance to epidermoid carcinoma.

Etiology and Pathogenesis of Chronic Murine Pneumonia

The history of work on the etiology of chronic murine pneumonia is reminiscent of that on distemper in dogs. For years, the murine disease was commonly contended (or assumed) to be of bacterial origin, although the exact etiology was uncertain, and its pathogenesis was not clear. The most extensive investigations have been those of Nelson[1,12-17,19-20] at the Rockefeller Institute, New York, who demonstrated that the disease was not primarily bacterial in origin, and has shown recently that the disorder is initiated in the young rat by a virus. Saxton and Kimball[18] suggested that the cause is not directly or indirectly related to dietary deficiency factors. It seemed, however, that a comparison of rats on a standard cube diet should be made with animals raised on a relatively simple but adequate diet. To this end, 100 rats were weaned at 21 days and divided into two groups. One group received our standard commercial cube diet and the other received bread and milk with weekly supplements of cod-liver oil. All animals were sacrificed at the age of 4 months and examined. Our findings indicated no significant differences as far as the lungs are concerned. However, it is worth mentioning that the rats fed bread and milk were beautiful smooth-coated animals; they were larger on the average but not obese, cleaner, and more active than the animals fed the standard diet.

Many attempts, over the past 40 years, have been made to prove a specific bacterial origin and a variety of microorganisms have been isolated, a few of which might be listed: a diphtheroid (*Bacillus muris*), a Gram-negative streptothrix (which was probably *Streptobacillus moniliformis*), *Br. bronchiseptica* (also once thought to be the cause of canine distemper), *Pasteurella muricida*, and *Streptobacillus moniliformis* (*Actinobacillus actinoides*).

Much work also has been carried out along the same lines by Miss

K. Wilson in the Bacteriology Section of our own laboratories, and the following is a brief summary of her findings. Reference has been made that in one epizootic outbreak, in which a large number of deaths occurred, type II pneumococci were isolated from the heart's blood and pulmonary lesions. After the epizootic subsided, 70 per cent of the survivors were found to be carrying the same organism in their throats. Six months later, in another outbreak, the predominating organism recovered was Pasteurella, although from some rats type II pneumococci also were isolated. The rats at that time were housed in the same room as rabbits, which are notoriously susceptible to pasteurellosis, and may have been the origin of the murine infection. Cultures were made also from 43 rats of varying ages from 5 weeks to over 1 year and a variety of organisms recovered, among which were *Br. bronchiseptica* (12 times), *Pasteurella multocida* (4 times), a diptheroid, staphylococci, and a hemolytic streptococcus, but no pleuropneumonia-like organisms at any time. The lungs of the very young rats were relatively free from pathogenic organisms.

Because of the high carrier rate and lack of more positive evidence, an etiologic relationship remained unsubstantiated. Without exception, these organisms (in the experience of other workers) were not capable of invoking the disease experimentally in its entire chronicity. It became apparent that the lesion was initiated by some other factor and then was followed by secondary bacterial invaders, for none of the bacteria isolated fulfilled the requirements of the original postulates of Koch. One great handicap in critical experimental study patently stemmed from the lack of rat colonies free from the natural disease.

The confusion of the earlier work on etiology has been clarified by Nelson.[16,19,20] He demonstrated that there are two independent diseases involved in the chronic respiratory complex of rats by eradicating one—the infectious catarrh—without an effect on the incidence of the chronic pulmonary disease.[17] The infectious catarrh, also a common disorder of the mouse, has been shown by Klieneberger and Steabben[21]* to be caused by pleuropneumonia-like organisms. This disease, occurring in young and adult rats, is of slow onset, long duration, and may involve the middle ear, occasionally with contiguous infection of the inner ear (labyrinthitis), which is manifested by circling and twisting in the living rat. In 1946, Nelson[14] presented experimental proof that the primary factor in chronic pulmonary disease of rats was a virus. He showed that the disease was transmissible

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* Reference 1 also may be consulted.

to mice by a filterable suspension of lungs from affected rats, that the agent was effective at dilutions of 10^7, that it was removed by centrifugation at 9,000 r.p.m. for 30 minutes, and that, although it could not be cultivated in chick embryos, it remained active for 3 months in the frozen state (i.e., as long as it was kept). The infection was established regularly in mice by nasal instillation, and by direct contact, but bronchiectasis was not produced in mice kept as long as 32 weeks. The virus was demonstrated in practically all breeders, and was transmitted by the female to her young soon after their birth, infectivity being possible throughout the life span of the rat. There is no doubt that these two infections have coexisted in most breeding colonies (England and the United States), so that there can be infectious catarrh and chronic pneumonia or a combination in most colonies. More recently, Nelson has found that the mouse carries a similar virus naturally.[22]

The pathogenesis still is not clear, particularly in regard to bronchiectasis. It has been postulated by some that the bronchiectasis is due to plugging of the bronchi by mucus followed by the growth of microorganisms.[23] Others have felt that mucus secretion is a result, not the cause, of bronchial obstruction, the latter being initiated by massive lymphoid aggregates to the extent of forming polypoid masses circumscribing and constricting the bronchial lumen.[8] Thus it is that a combined viewpoint of obstruction and bacterial infection has become accepted as an explanation of bronchiectasis. Our contention, however, is that this concept does not explain adequately the production of bronchiectasis, because we have not been impressed that lymphoid proliferation, even in its most massive proportions, significantly constricts the bronchial lumen. This process may promote stasis by increasing the rigidity of the bronchial tree, but such stasis, uncomplicated by any other factors, would not be expected to produce more than very moderate dilatation of bronchi.

Patchy interstitial pneumonitis, occurring early in the course of chronic pulmonary disease, was mentioned earlier, and this is often a salient change in alveoli contiguous to the bronchial tree, accompanied by varying degrees of atelectasis. What one frequently observes then, particularly in the arborizations of the bronchial tree beyond the primary bronchus, is a process of lymphoid infiltration of the bronchial wall accompanied by peribronchial pneumonitis and atelectasis. The sequence of this is peribronchial fibrosis, and the picture produced is that of early bronchiectasis. The factor of stasis is now exemplified by a somewhat dilated structure with a swollen (hypertrophied) epithe-

lium, in which ectasia continues with concomitant increasing ate-
lectasis. The chronic disease, as we know it, is thus a slowly progressive
process over many months. This progression of tissue changes is more
in keeping with those leading to bronchiectasis in man.*

Establishment of Rat Colonies Free from Chronic Murine Pneumonia

All workers who have been in charge of rat breeding colonies recog-
nize the inherent difficulties in controlling chronic pulmonary disease.
The work of Nelson[1,6,12-17,19,20,22] showed why some of those difficulties
existed, in spite of possibly excellent conditions as to housing, cage
design, diet and watering systems, animal husbandry, and the person-
nel handling the animals. None of these factors can have any direct
significant influence on the presence of viral or other infections of such
low virulence and protracted chronicity. Nevertheless, they are funda-
mental for the maintenance of an otherwise healthy colony. From
personal experience (J.R.M.I.), none of the modern chemotherapeutic
agents has a demonstrable prophylactic or therapeutic value. While
prophylactic therapeutic measures by drugs might be possible in small
colonies, they would be impractical for large ones with a production
goal of more than 50,000 rats a year, unless medication were possible
in drinking water or pelleted diet. In rats to be used for the study of
experimental infection, such measures would be unwarranted and un-
desirable. Total eradication must be achieved by other measures, such
as those proposed by Nelson.

Nelson[6] (1951) reported that, starting with young breeders, ob-
tained by caesarian section and then hand-fed, a breeding colony of
rats (not a germ-free one) was established for 2 years with absence of
virus infection, pleuropneumonia-like organisms, and *Streptobacillus
moniliformis*. Examination of adult year-old rats from this colony
showed no evidence of disease in the lungs, middle ears, or nasal pas-
sages. Young rats from this colony were shown to be susceptible to
the murine pneumotropic virus, with lesions induced identical to those
of naturally occurring chronic pneumonitis. We suggest, if the breed-
ing system of Nelson is tried, that a number of small autonomous units
might be better than a single large one, because if disease prevention
breaks down in one unit, then all is not lost. Finally, in such an oper-
ation, all principles, well known and proved by experience to be neces-
sary for the successful large-scale breeding of any laboratory species,
should be rigidly adhered to, viz., those concerned with nutrition, cage

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* We realize that our descriptions almost certainly cover the chronic effects of both in-
fectious catarrh and murine pneumonia.

sterilization, disease prevention, adequate ventilation, and record-keeping; all animal colonies should be quarantined to avoid possible contact with infection from wild vermin, and should have no physical proximity to experimental animals.

SUMMARY

Acute epizootic infections in laboratory rats are relatively rare. Chronic murine pneumonia, on the other hand, is a perennial problem. With a high morbidity but low mortality, it is of considerable significance in experimental studies on the lungs, particularly in assessing the pathologic effects of toxic materials. The complex of chronic lesions may render the task of differentiating naturally from experimentally acquired lesions extremely difficult. The incidence of the clinically silent lesions reported in the literature ranges from 50 to 75 per cent of rats which appeared in normal health. Our figures are higher due to a more critical pathologic examination. The macroscopic and histologic appearances of the chronic pneumonia complex are described. Exception is taken to previous views on the pathogenesis of the lesion and consideration is given to recent studies by others on the etiology of the disease, particularly to its probable viral origin.

ADDENDUM BY THE SENIOR AUTHOR

Establishment of Pulmonary Disease-Free Colony of Rats

After this paper was completed, an attempt was made to produce a colony of rats free from chronic murine pneumonia. Through the kindness of Dr. John B. Nelson (Rockefeller Institute, New York), 56 rats, 1 to 3 months old, were obtained on April 4, 1955. Eleven paired matings were set up. No attempt was made at establishing fertility records. By October 1 nearly 100 rats from the first, second, or third generation had been supplied the Armed Forces Institute of Pathology, Washington, D.C., where a separate colony of brother-sister matings has been started under Lt. Col. T. C. Jones, V.C.

The animals were not kept under strict isolation. All rats were kept on wire without bedding; paper was provided to pregnant animals for nests; the usual principles of hygiene and sterilization of cages were adopted; standard pelleted diets were given with a supplement of fresh tinned dog food to pregnant "pairs." Routine mortality of the progeny, which were weaned until October and which were over 1 day old, was almost nil. All original parents (the 11 pairs) were killed when about 9 months old, and when at least three or four litters had been born from each female. Many of the progeny now have been examined. Of the remaining rats from Dr. Nelson, 8 were killed at 3

months, and 26 at 6 and 7 months of age. Thorough routine necropsies and other examinations were made of all rats. The results were as follows:

The rats were free from Salmonella as determined by bacteriologic examination of feces, and were practically free from gastro-intestinal helminthic infestation. No middle ear infection occurred. Splenectomy of some rats revealed an absence of Bartonella infection. Examination of pieces of skeletal muscle, esophagus, and heart showed an absence of sarcosporidia and no myositis or myocarditis of any kind. In not a single rat were lesions found, macroscopically or microscopically, which bore resemblance to any feature of the complex of chronic pneumonia. The absence of peribronchial lymphoid tissue, except microscopic collections, supported our belief that such tissue is not a "normal species histologic difference for rats." (We think lymphoid proliferation in the rat lung may be the initial response to the Nelson rat pneumonia virus.) Bacteriologic examinations were made of the left pulmonary lobe of all rats (the four right lobes being reserved for histologic study); no organisms were recovered which were of undisputed pathogenic importance, and neither pleuropneumonia-like organisms nor *Br. bronchiseptica* were isolated (both species have been recovered many times within recent months from our "normal" stock colony).

We conclude that it is not a difficult matter to raise *"normal rats"* in a normal environment free from infections, particularly those which affect the lungs and are therefore of importance to workers studying toxic inhalants. There are no intrinsic difficulties in raising a disease-free rat colony. It costs no more to do so in terms of time, labor, and food. Further, compared to the mouse, the rat is an amazingly resistant animal.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Lung, rat. A vein within the parenchyma shows striated cardiac muscle fibers in the adventitia. Weigert's elastic method. $\times$ 85.

FIG. 2. Lung, rat, 6 months old. Massive lymphoid aggregates around bronchi with disruption of the walls. Hematoxylin and eosin stain. $\times$ 35.

FIG. 3. Lung, rat, 2 months old. Low-power view of broad, irregular sleeves of lymphoid tissue outlining the bronchial tree. Hematoxylin and eosin stain. $\times$ 4.

FIG. 4. Lung, rat. Focal collections of foamy macrophages in the alveolar spaces of an otherwise normal structure. Hematoxylin and eosin stain. $\times$ 435.

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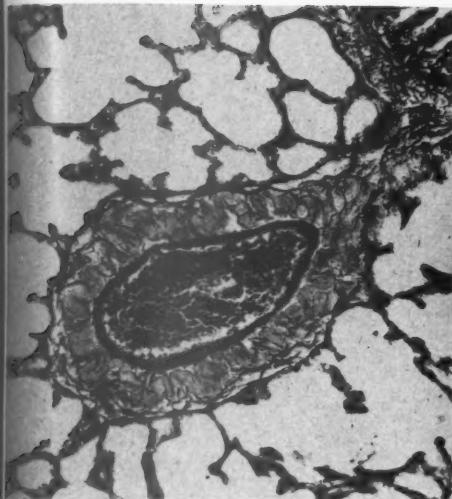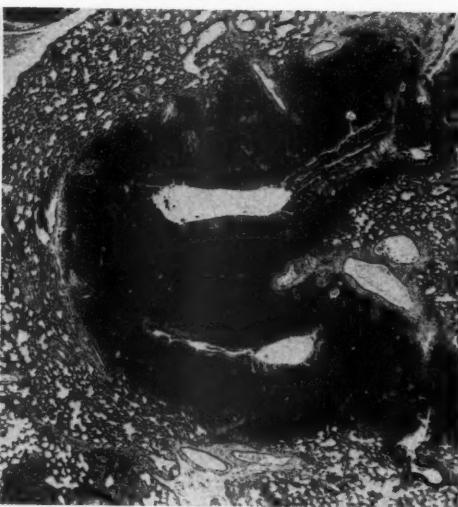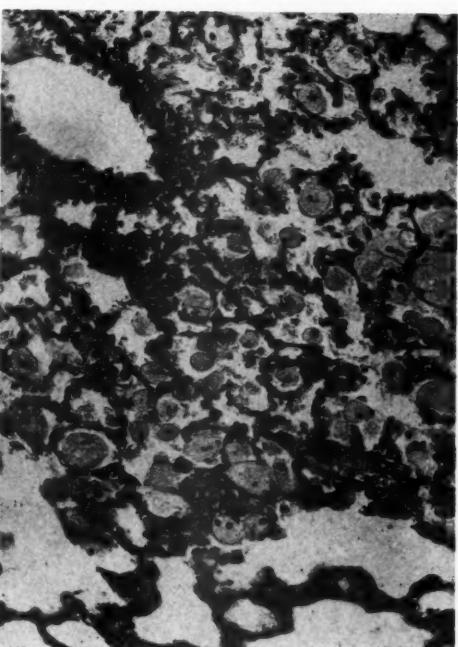
FIG. 5. Lung, rat. Complete induration of right apical and middle lobes and apex of left lung; scattered miliary lesions in other lobes.

FIG. 6. Lungs, rat, 3 months old. Multiple lesions representing what appears to be a fulminating bronchiectasis; infrequent, but occurs in young animals.

FIG. 7. Lung, rat, 3 months old. Perivascular cuffing. Hematoxylin and eosin stain. × 85.

FIG. 8. Lung, rat, 3 months old. An area of interstitial pneumonitis. Hematoxylin and eosin stain. × 345.

FIG. 9. Lung, rat. Typical lesion of chronic pneumonia showing complete atelectasis of a lobe. Hematoxylin and eosin stain. × 35.

FIG. 10. Lung, rat. Advanced lesion of chronic pneumonia; all right lobes are involved in bronchiectasis. Hematoxylin and eosin stain. × 4.

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